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(54) Title: IMPROVED EXPRESSION OF CRY3B INSECTICIDAL PROTEIN IN PLANTS		
(57) Abstract <p>The present invention discloses methods and compositions comprising a group of novel expression cassettes which provide significantly improved levels of accumulation of Coleopteran inhibitory Cry3B and Cry3B variant amino acid sequences when these are expressed in plants. The preferred embodiments of the invention provide at least up to ten fold higher levels of insect controlling protein relative to the highest levels obtained using prior compositions. In particular, transgenic maize expressing higher levels of a protein designed to exhibit increased toxicity toward Coleopteran pests deliver superior levels of insect protection and are less likely to sponsor development of populations of target insects that are resistant to the insecticidally active protein.</p>		

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IMPROVED EXPRESSION OF CRY3B INSECTICIDAL PROTEIN IN PLANTS

1.0 BACKGROUND OF THE INVENTION

1.1 FIELD OF THE INVENTION

5 The present invention discloses transgenic plants expressing substantially higher levels of insect controlling *Bacillus thuringiensis* δ -endotoxin. Methods for obtaining such plants and compositions, and methods for using such plants and compositions are described. Also disclosed are improved polynucleotide cassettes containing preferred protein coding sequences which impart the substantially higher levels of insect controlling δ -endotoxins. The preferred embodiments of the invention surprisingly provide up to ten fold higher levels of insect
10 controlling protein relative to the highest levels obtained using prior compositions. In particular, transgenic maize expressing higher levels of a protein designed to exhibit increased toxicity toward Coleopteran pests deliver superior levels of insect protection and are less likely to sponsor development of populations of target insects that are resistant to the insecticidally active
15 protein.

1.2 DESCRIPTION OF THE RELATED ART

 Almost all field crops, plants, and commercial farming areas are susceptible to attack by one or more insect pests. Particularly problematic are Coleopteran and Lepidopteran pests.
20 Because crops of commercial interest are often the target of insect attack, environmentally-sensitive methods for controlling or eradicating insect infestation are desirable. This is particularly true for farmers, nurserymen, growers, and commercial and residential areas which seek to control insect populations using ecologically friendly compositions.

 The most widely used environmentally-sensitive insecticidal formulations developed
25 in recent years have been composed of microbial protein pesticides derived from the bacterium *Bacillus thuringiensis*, a Gram-positive bacterium that produces crystal proteins or inclusion bodies which are specifically toxic to certain orders and species of insects. Many different strains of *B. thuringiensis* have been identified which produce one or more insecticidal crystal proteins as well as other insecticidal non-crystal forming proteins. Compositions including *B.*
30 *thuringiensis* strains which produce insecticidal proteins have been commercially available and used as environmentally acceptable insecticides because they are quite toxic to specific target insect pests, but are harmless to plants and to vertebrate and invertebrate animals. More

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importantly, because these insect controlling proteins have to be ingested by susceptible target insect pests in order to exert their insecticidal or toxic effects, judicious application of such protein compositions limits or prevents non-target insect members of the susceptible order which may also be susceptible to the composition from significant exposure to the proteins (for example, non-target Lepidopteran species where Lepidopteran specific B.t. crystal protein is used in an insecticidal formulation). Additionally, insects of various orders have been shown to totally lack susceptibility to specifically targeted insecticidal proteins even when ingested in large amounts.

1.2.1 δ -ENDOTOXINS

δ -endotoxins are used to control a wide range of plant-eating caterpillars and beetles, as well as mosquitoes. These proteins, also referred to as insecticidal crystal proteins, crystal proteins, and Bt toxins, represent a large collection of insecticidal proteins produced by *B. thuringiensis* that are toxic upon ingestion by a susceptible insect host. Over the past decade research on the structure and function of *B. thuringiensis* toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. A recent review describes the genetics, biochemistry, and molecular biology of Bt toxins (Schnepf et al., *Bacillus thuringiensis* and its Pesticidal Crystal Proteins, Microbiol. Mol. Biol. Rev. 62:775-806, 1998). Based on the accumulated knowledge of *B. thuringiensis* toxins, a generalized mode of action for *B. thuringiensis* toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial digestion by gut specific proteases catalyzing specifically a cleavage at a peptide site within a protoxin structure which "activates" the toxin, binding of the toxin to the midgut cells' brush border, formation of a pore in the insect midgut cell, and the disruption of cellular homeostasis (English and Slatin, 1992).

1.2.2 GENES ENCODING CRYSTAL PROTEINS

Many of the δ -endotoxins are related to various degrees by similarities in their amino acid sequences. Historically, the proteins and the genes which encode them were classified based largely upon their spectrum of insecticidal activity. A review by Höfte and Whiteley

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(1989) discusses the genes and proteins that were identified in *B. thuringiensis* prior to 1990, and sets forth the nomenclature and classification scheme which has traditionally been applied to *B. thuringiensis* genes and proteins. The original nomenclature took advantage of the discovery that the few Bt Cry proteins known at the time generally fell into a limited number of classes, wherein each class represented proteins having specificity for specific orders of insects. For example, *cry1* genes encoded Lepidopteran-toxic Cry1 proteins. *cry2* genes encoded Cry2 proteins that were generally toxic to both Lepidopterans as well as to Dipterans. *cry3* genes encoded Coleopteran-toxic Cry3 proteins, while *cry4* genes encoded Dipteran-specific toxic Cry4 proteins. The nomenclature has, for the past decade or more become rather confusing with the discovery of more distantly related classes of insecticidal Bt proteins. More recently, a simplified homogeneous nomenclature and basis for classifications of Bt proteins has been adopted and has been reviewed by Schnepf et al. (1998). Schnepf et al. (1998) also provides a structural solution for a Cry1 crystal. This simplified nomenclature will be adopted herein. The convention of identifying Bt genes with lower case, italicized letters (eg. *cry1Ab1*) and identifying Bt proteins with uppercase first character (eg. Cry1Ab1) will also be observed herein

Based on the degree of sequence similarity, the proteins have been further classified into subfamilies. Proteins which appeared to be more closely related within each family were assigned divisional letters such as Cry1A, Cry1B, Cry1C, etc. Even more closely related proteins within each division were given names such as Cry1Ca, Cry1Cb, etc. and still even more closely related proteins within each division were designated with names such as Cry1Bb1, Cry1Bb2, etc.

The modern nomenclature systematically classifies the Cry proteins based upon amino acid sequence homology rather than upon insect target specificities. The classification scheme for many known toxins, not including allelic variations in individual proteins, is summarized in regularly updated tables which can be obtained from Dr. Neil Crickmore at http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html.

1.2.3 BIO-INSECTICIDE POLYPEPTIDE COMPOSITIONS

The utility of bacterial crystal proteins as insecticides was extended beyond Lepidopterans and Dipteran larvae when the first isolation of a Coleopteran-toxic *B.*

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thuringiensis strain was reported (Krieg *et al.*, 1983; 1984). This strain (described in U.S. Patent 4,766,203, specifically incorporated herein by reference), designated *B. thuringiensis* var. *tenebrionis*, was reported to be toxic to larvae of the Coleopteran insects *Agelastica alni* (blue alder leaf beetle) and *Leptinotarsa decemlineata* (Colorado potato beetle).

5 U. S. Patent 5,024, 837 also describes hybrid *B. thuringiensis* var. *kurstaki* strains which showed activity against Lepidopteran insects. U. S. Patent 4,797,279 (corresponding to EP 0221024) discloses a hybrid *B. thuringiensis* containing a plasmid from *B. thuringiensis* var. *kurstaki* encoding a Lepidopteran-toxic crystal protein-encoding gene and a plasmid from *B. thuringiensis tenebrionis* encoding a Coleopteran-toxic crystal protein-encoding gene. The
10 hybrid *B. thuringiensis* strain produces crystal proteins characteristic of those made by both *B. thuringiensis kurstaki* and *B. thuringiensis tenebrionis*. U. S. Patent 4,910,016 (corresponding to EP 0303379) discloses a *B. thuringiensis* isolate identified as *B. thuringiensis* MT 104 which has insecticidal activity against Coleopterans and Lepidopterans. More recently, Osman et al. disclosed a natural *Bacillus thuringiensis* isolate which displayed activity against at least two
15 orders of insects and against nematodes (WO 98/30700).

It has been known for more than two decades that compositions comprising Bt insecticidal proteins are effective in providing protection from insect infestation to plants treated with such compositions. More recently, molecular genetic techniques have enabled the expression of Bt insecticidal proteins from nucleotide sequences stably inserted into plant
20 genomes (Perlak et al., Brown & Santino, etc.). However, expression of transgenes in plants has provided an avenue for increased insect resistance to Bt's produced in plants because plants have not been shown to produce high levels of insecticidal proteins. It was initially believed that gross morphological or topological differences in gene structure and architecture between plant and bacterial systems was the limitation which prevented over-expression of Bt transgenes in
25 plants. These differences were seemingly overcome as disclosed by Perlak et al. (US Patent No. 5,500,365) and by Brown et al. (US Patent No.'s 5,424,412 and 5,689,052) wherein transgenes encoding Bt insecticidal protein which contained plant preferred codons were shown to improve the levels of expression. Alternatively, truncating the protoxin coding domain to the shortest peptide coding domain which still encoded an insecticidal protein was also deemed sufficient to
30 overcome the limitation of vanishingly low expression levels of the Bt encoding transgene in planta. Expression levels of Bt proteins *in planta* from transgenes has varied widely independent

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of the means used for expression, and accumulated protein levels have ranged from virtually undetectable to 2 parts per million to around 20 to 30 parts per million. However, even though all of these approaches provided improved levels of Bt protein accumulation in plants, none provided levels of expression which could ensure that insect resistance would not become a problem without the necessity of coordinate expression of one or more additional insecticidal toxins by the transgenic plant, or alternatively without the coordinate topical application of additional supplemental Bt or insecticidal chemical compositions.

The importance of accumulation of higher levels of Bt toxin for preventing insect resistance to individual Bt toxins has been understood for some time. Various laboratory studies in which selection against Bt was applied over several generations of insects have confirmed that resistance against Bt insecticidal proteins is seldom obtained. It should be emphasized that laboratory conditions represent rather low but constant selection pressure conditions, allowing for the survival of a sub-population of insects which have been subjected to insecticidal pressure and which produce the subsequent generations of insects. Succeeding generations are also maintained on media containing low but constant concentrations of insecticidal protein. Generally, concentrations used for selection pressures range from LC40 to around LC60 or so, however, LC95 concentrations have also tested for the development of resistance. In most cases, resistance is acquired slowly, generally developing within a reasonably few generations, for example 10-50 generations. However, such resistance is not observed where substantially higher levels of toxin are used, or in situations in which multiple toxins are provided.

At present, recombinant plants expressing commercially useful levels of Bt insecticidal protein generally contain only one gene encoding a single class of Bt. Such plants are anticipated to have a very limited duration of use for two reasons. First, these plants are expressing insufficient levels of the insecticidal protein to ensure that all target insects exposed to and feeding from the plant tissues will succumb due to the dose of toxin ingested. Second, because of the insufficient insecticidal protein levels, the potential for development of resistance is unreasonably increased. This is not to say that the level of toxin produced by such transgenic plants is insufficient to be effective. This merely represents the limitations of expression of δ -endotoxins in planta even when using sequences encoding Bt δ -endotoxin which have been modified to conform to plant preferred sequences. One limitation which has been observed for many Bt δ -endotoxin encoding sequences modified for expression in plants is that is has been

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impossible to predict which Bt δ -endotoxin would be effective for expression in plants. (For example, expression of Cry2Aa in cotton plants results in phytotoxicity when targeted to the chloroplast, however expression of a closely related *cry2Ab* sequence is not phytotoxic when targeted to the chloroplast. (Corbin et al., US Patent Application, Serial No. 09/186,002). Even
5 so, levels of δ -endotoxin protein produced in plants is not sufficient to be effective against all desired target insect species known to be susceptible to a given type and class of δ -endotoxin.

As indicated above, alternative approaches to development of resistance to insecticidal proteins has included ineffective attempts to increase the expression levels of transgenes in plants. Alternatively, additional insecticidal genes could be engineered into plants
10 so that multiple toxins are coordinately expressed. This would provide a more effective means for delaying the onset of resistance to any one combination of Bt's, however, this still does not overcome the limitation of insufficient levels of insecticidal protein accumulating in the recombinant plant(s). An additional alternative to insufficient levels of expression has been to engineer genes encoding Bt insecticidal crystal proteins which demonstrate improved insecticidal
15 properties, having either a broader host range or an increased biological activity, which could conceivably result in requiring less of the recombinant protein to control a target insect species than was required of the native form of the protein.

The combination of structural analyses of *B. thuringiensis* toxins followed by an investigation of the function of such structures, motifs, and the like has taught that specific
20 regions of crystal protein endotoxins are, in a general way, responsible for particular functions.

Domain 1, for example, from Cry3Bb and Cry1Ac has been found to be responsible for ion channel activity, the initial step in formation of a pore (Walters *et al.*, 1993; Von Tersch *et al.*, 1994). Domains 2 and 3 have been found to be responsible for receptor binding and insecticidal specificity (Aronson *et al.*, 1995; Caramori *et al.*, 1991; Chen *et al.* 1993; de Maagd
25 *et al.*, 1996; Ge *et al.*, 1991; Lee *et al.*, 1992; Lee *et al.*, 1995; Lu *et al.*, 1994; Smedley and Ellar, 1996; Smith and Ellar, 1994; Rajamohan *et al.*, 1995; Rajamohan *et al.*, 1996; Wu and Dean, 1996). Regions in domain 2 and 3 can also impact the ion channel activity of some toxins (Chen *et al.*, 1993, Wolfersberger *et al.*, 1996; Von Tersch *et al.*, 1994).

Unfortunately, while many investigators have attempted, few have succeeded in
30 making mutated crystal proteins with improved insecticidal toxicity. In almost all of the examples of genetically-engineered *B. thuringiensis* toxins in the literature, the biological

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activity of the mutated crystal protein is no better than that of the wild-type protein, and in many cases, the activity is decreased or destroyed altogether (Almond and Dean, 1993; Aronson *et al.*, 1995; Chen *et al.*, 1993, Chen *et al.*, 1995; Ge *et al.*, 1991; Kwak *et al.*, 1995; Lu *et al.*, 1994; Rajamohan *et al.*, 1995; Rajamohan *et al.*, 1996; Smedley and Ellar, 1996; Smith and Ellar, 1994; Wolfersberger *et al.*, 1996; Wu and Aronson, 1992). However, Van Rie et al. have recently accomplished the improvement of a Cry3A δ -endotoxin having increased Coleopteran insecticidal activity by identifying a single mutant having increased insecticidal activity. Van Rie et al. propose a method for identifying mutants having increased insecticidal activity in which the method consists of identifying amino acid mutations which decrease the insecticidal activity, and selectively altering those residues by site directed mutagenesis to incorporate one or more of the naturally occurring 20 amino acids at those positions, and feeding the various forms of the resulting altered protein to western or northern corn rootworms to identify those having improved activity (US Patent 5,659,123). While no sequences were enabled using the method, as mentioned above, Van Rie et al. succeeded in identifying only one sequence having increased activity and did not demonstrate an increase in expression of the mutant form as compared to the native sequence.

For a crystal protein having approximately 650 amino acids in the sequence of its active toxin, and the possibility of 20 different amino acids at each position in this sequence, the likelihood of arbitrarily creating a successful new structure is remote, even if a general function to a stretch of 250-300 amino acids can be assigned. Indeed, the above prior art with respect to crystal protein gene mutagenesis has been concerned primarily with studying the structure and function of the crystal proteins, using mutagenesis to perturb some step in the mode of action, rather than with engineering improved toxins.

Collectively, the limited successes in the art to develop non-naturally occurring toxins with improved insecticidal activity have stifled progress in this area and confounded the search for improved endotoxins or crystal proteins. Rather than following simple and predictable rules, the successful engineering of an improved crystal protein may involve different strategies, depending on the crystal protein being improved and the insect pests being targeted. Thus, the process is highly empirical.

Accordingly, traditional recombinant DNA technology is clearly not routine experimentation for providing improved insecticidal crystal proteins. What has been lacking in the prior art are rational methods for

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producing genetically-engineered *B. thuringiensis* crystal proteins that have improved insecticidal activity and, in particular, improved toxicity towards a wide range of Lepidopteran, Coleopteran, or Dipteran insect pests. Methods and compositions which address these concerns were disclosed in US Patent Application No. 08/993,170 (December 18, 1997; English et al.) and other related US applications (08/993,722, Dec. 18, 1997, English et al.; 08/993,755, Dec. 18, 1997, English et al.; and 08/996,441, Dec. 18, 1997, English et al.) and in Van Rie et al. (US Patent 5,659,123, Jun 1, 1999). In addition, recombinantly improved δ -endotoxins have continued to be expressed poorly and/or cause phytotoxic effects when expressed in plants, thus leading to the recovery of fewer commercially useful transgenic events.

2.0 Summary of the Invention

Described herein are novel compositions and methods for expressing in transformed plants variant Cry3 *B. thuringiensis* δ -endotoxins having significant Coleopteran inhibitory activity. These compositions and methods advantageously result in plants expressing *B. thuringiensis* Cry3 δ -endotoxins at increased levels not previously observed for Cry δ -endotoxins. Increased levels of Cry3 δ -endotoxin expression are reflected in the attainment of higher maximal expression levels in individual transgenic insertion events. Unexpectedly, the particular compositions disclosed herein result in the recovery of an increased percentage of transgenic events which manifest expression levels that far exceed threshold levels of expression necessary for Coleopteran insect control and which provide sufficient toxin levels capable of supporting a resistance management strategy. Since Cry3 δ -endotoxins are typically less potent than other δ -endotoxins commonly used to control Lepidopteran or Dipteran target pests when expressed in transgenic plants, attainment of higher maximal levels of Cry3 δ -endotoxin expression and recovery of more transgenic events with effective expression levels are both critical in isolating transgenic events expressing Cry3 δ -endotoxin which exhibit commercially useful levels of target insect control.

Another limitation of the prior art addressed by the present invention is the development of insect resistance to δ -endotoxins provided by plant expression. Specifically, the instant invention provides a superior strategy for the delay or elimination of the development of resistance to Cry3 δ -endotoxins through improved accumulation of δ -endotoxin within plant cells so that levels of the δ -endotoxin are maintained in-planta above a threshold level of protein, typically measured in parts per million (ppm). Improved expression of δ -endotoxins, which also

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should be taken to mean increased expression in view of what has been previously observed in the art, is believed to result in delayed onset of insect resistance and thus extends the utility of plant expressed δ -endotoxins as insect control agents.

In preferred embodiments, the present invention provides isolated and purified novel
5 Cry3B δ -endotoxin proteins exhibiting particularly effective insecticidal activity directed toward controlling Coleopteran pest insect species. Such δ -endotoxin proteins of the present invention are provided by expression from isolated, purified and improved or enhanced DNA or polynucleotide sequences each comprising a Cry3 δ -endotoxin coding sequence placed under the control of preferred plant functional gene expression elements such as a promoter, an
10 untranslated leader sequence, an intron and a transcription termination and polyadenylation sequence. Some preferred DNA or polynucleotide sequences may also provide for plastid or chloroplast targeting protein sequences. Preferred DNA constructs of the present invention include those constructs which encode Cry3 δ -endotoxins exhibiting Coleopteran-inhibitory or Coleopteran-controlling activity. In an illustrative embodiment, polynucleotide sequences are
15 assembled into an expression cassette for introduction into plant genomic DNA, wherein the expression cassette comprises a Cry3Bb δ -endotoxin variant coding sequence operably linked to a sequence comprising a promoter, an untranslated leader sequence, an intron and a transcription termination and polyadenylation sequence. In particular, a transgene localized within a plant operable polynucleotide expression cassette or polynucleotide sequence comprising an
20 expression cassette which is comprised of genetic elements which function in plant cells to express a desired protein from a nucleic acid coding sequence (the transgene) which is operably localized within said expression cassette. The coding sequence is linked upstream to at least a promoter sequence, an untranslated leader sequence (UTL), an intron sequence, and in-frame in certain indicated embodiments to a sequence encoding a plastid or chloroplast targeting peptide.
25 The coding sequence is also linked downstream to at least a plant functional transcription termination and polyadenylation sequence. Polynucleotide sequences comprising such an expression cassette are shown herein to improve expression of the desired protein encoded from within the cassette, improve the number of events obtained from the use of the polynucleotide sequence in plant transformation, wherein said improved number of events contain the desired
30 transgene localized within the expression cassette and exhibit improved levels of expression of

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one or more desired proteins. The improved number of events are also surprisingly observed to express the desired protein at levels above 2 to 5 parts per million but in general below 200 to 500 parts per million of total cell protein. Even more surprising were some events in particular which expressed the desired protein at levels well above 500 ppm. Indicated embodiments

disclose a sequence encoding a variant Cry3Bb δ -endotoxin comprising the isolated and purified SEQ ID NO:9, from *Nco*I to *Eco*RI as set forth in Figure 1 illustrating plasmid pMON25096.

Yet other embodiments disclose a variant Cry3Bb δ -endotoxin coding sequence comprising an isolated and purified SEQ ID NO:11, from *Nco*I to *Eco*RI as set forth in Figure 2 illustrating plasmid pMON33741. It is contemplated, however, that any Cry3 δ -endotoxin exhibiting

substantial Coleopteran-inhibitory or Coleopteran-controlling activity greater than or equal to that disclosed in the present invention could be utilized according to the embodiments of the present invention, with those Cry3 proteins bearing substantial homologies to Cry3Bb being particularly preferred.

In a preferred embodiment, the invention provides for transgenic plants which have been transformed with a DNA construct or expression cassette of the present invention that is expressed and translated at unexpectedly high levels by the plant which results in surprisingly high levels of δ -endotoxin accumulation. Monocotyledenous plants may be transformed according to the methods and with the DNA constructs disclosed herein. However, it is also anticipated that dicotyledenous plants could also be transformed with DNA sequences disclosed herein by one skilled in the art in order to obtain transgenic plants providing unexpectedly useful levels of insect resistance without the risk of development of insect resistance to the δ -endotoxin. The plant transformed by the instant invention may be prepared, in a further preferred embodiment, by a process including obtainment of the isolated and purified DNA construct contained within the expression cassette, and then transforming the plant with the construct so that the plant expresses the protein for which the construct encodes. Alternatively, the plant transformed by the instant invention may be prepared, in a further preferred embodiment, by a process including introduction of the isolated and purified DNA construct into a transformation competent *Agrobacterium* strain, and then transforming the plant with the *Agrobacterium* strain containing the construct so that the plant expresses the proteins for which the construct encodes. It has been observed herein that transformation of plants by the disclosed compositions and

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methods results surprisingly in increased frequencies of transformants exhibiting transgene expression as well as in the recovery of individual transgenic events exhibiting unexpectedly higher absolute levels of transgene expression.

It is contemplated that the increased expression levels observed in the disclosed invention will

5 allow for reduced development of insect resistance to Bt δ -endotoxins presented to target insect pests. This may be achieved by transforming a plant with the preferred DNA construct to achieve high rates of Cry3 expression alone, or by simultaneously exposing target insects to the disclosed Cry3 δ -endotoxins along with other compositions effective in controlling Coleopteran species such as variants of Cry3B (English et al., WO 99/31248), variant Cry3A or variant
10 Cry3D (US Patent 5,659,123), CryET33 and CryET34 (Donovan et al., WO 97/17600), CryET70 (US Application Serial No. 09/184,748; Mettus et al., November 2, 1998), Cry6A, Cry6B, Cry8B (US Pat. No. 5,277,905), CryET29 (Rupar et al., WO 97/21587), insecticidal acyl lipid hydrolases, combinations of amino acid oxidases and tedanalactam synthases (Romano et al., US Application Serial No. 09/063,733, filed April 21, 1998), or insecticidal proteins such as
15 VIP1 (Gay, WO 97/26339; Gourlet et al., WO 98/02453) and VIP3 (Estruch et al., US Pat. No. 5,877,012; 1999) among others. Susceptible target insects include *Diabroticus* spp. Wire Worm in *Zea mays* and *Leptinotarsa decemlineata* (Say) in *Solanum tuberosum*, and Boll Weevil in *Gossypium* species (cotton).

It is therefore contemplated that the compositions and methods disclosed by the present
20 invention will provide many advantages over the prior art including those specifically outlined above. Other advantages include improved control of susceptible target insect pests and achieving season long protection from insect pathogens. An additional advantage of the present invention provides for reducing the number of transgenic events that have to be screened in order to identify one which contains beneficial levels of one or more insect controlling compositions.

25 The present invention also encompasses cells transformed with the DNA constructs disclosed herein. Also, transformation vectors such as plasmids, bacmids, artificial chromosomes, viral vectors and such are contemplated as elements for use in delivering the nucleotide compositions of the present invention into contemplated cells in order to obtain transformed host cells, both prokaryotic and eukaryotic, which express the δ -endotoxin proteins encoded by the novel DNA
30 construct disclosed herein. It is further contemplated that in some instances the genome of a transgenic plant of the present invention will have been augmented through the stable integration

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of an expression cassette encoding a Coleopteran inhibitory or controlling *B. thuringiensis* δ -endotoxin or variants thereof as described herein. Furthermore, more than one transgene encoding an insecticidal composition will be incorporated into the nuclear genome, or alternatively, into the chloroplast or plastid genome of the transformed host plant cell. It is envisioned that more than one polynucleotide encoding an insecticidal crystal protein will be incorporated into the genome of a plant cell and it may be desirable to have two or even more sequences encoding insecticidal or other plant beneficial proteins within the nucleotide sequences contained within the cell. Such recombinantly derived proteins may exist as precursors, pro-toxins, or as fusions of beneficial proteins linked by flexible amino acid linker sequences or by protease specific cleavage sequences well known in the art. Chimeras comprising fusions of insecticidal proteins are also envisioned. The offspring of transgenic plant host cells can be manipulated artificially to produce whole recombinant plants exhibiting improved insecticidal properties, and the recombinant nucleotide sequences are shown herein to be heritable. The heritability of the elements is a preferred aspect of this invention, so that the expression elements are able to be delivered to lineal descendants of the original transformed host plant cell, giving rise first to a stably transformed plant whose constituent cells express the desired transgene, albeit tissue specific expression can be selectively manipulated generally through the choice of plant operable promoter selected for use in a given expression cassette, as described above. Transformed plants give rise to seeds containing the heritable expression cassette, and the seeds thus give rise to plants in lineal fashion which contain the expression cassette, generally in Mendelian fashion, particularly when selfed according to well known methods in the art.

3.0 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates plasmid pMON25096.

Figure 2 illustrates plasmid pMON33741.

Figure 3 illustrates plasmid pMON25097.

Figure 4 illustrates plasmid pMON33748.

Figure 5 illustrates the nucleotide and amino acid sequence translation of a variant Cry3Bb.11098 insecticidal protein as shown in SEQID NO:9.

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Figure 6 illustrates the nucleotide and amino acid sequence translation of a variant Cry3Bb.11231 insecticidal protein as shown in SEQID NO:11.

4.0 DETAILED DESCRIPTION OF THE INVENTION

The following detailed description of the invention is provided to aid those skilled in the art in practicing the present invention. Even so, the following detailed description should not be construed to unduly limit the present invention as modifications and variations in the embodiments discussed herein may be made by those of ordinary skill in the art without departing from the spirit and scope of the present invention.

4.1 DEFINITIONS

The following words and phrases have the meanings set forth below.

Biological functional equivalents. As used herein such equivalents with respect to the insecticidal proteins of the present invention are peptides, polypeptides and proteins that contain a sequence or moiety exhibiting sequence similarity to the novel peptides of the present invention, such as Cry3Bb.11231, and which exhibit the same or similar functional properties as that of the polypeptides disclosed herein, including insecticidal activity. Biological equivalents also include peptides, polypeptides and proteins that react with, *i.e.* specifically bind to antibodies raised against Cry3Bb and that exhibit the same or similar insecticidal activity, including both monoclonal and polyclonal antibodies.

Combating or Controlling Insect Damage in an agricultural context refers to reduction of damage in relative units to a crop or plant part caused by infestation of an insect pest. More generally, this phrase refers to reduction in the adverse effects caused by the presence of an undesired insect in any particular location.

Event refers to a transgenic plant derived from one of the following:

1. the insertion of foreign DNA into one or more unique sites in the nuclear genomic DNA;
2. the insertion of foreign DNA into one or more unique sites in the plastid, chloroplast or mitochondrial genome;

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3. the introduction of a stable, heritable, epigenetic vector into the cytoplasm of a plastid, chloroplast, or mitochondria; or
4. a combination of any of the foregoing processes.

Events derived from these processes contain an expression cassette expressing a desired coding sequence as described herein. Events are also referred to as ITE's (independent transformation events).

Expression: The combination of intracellular processes, including transcription, translation, and other intracellular protein and RNA processing and stabilization functions, undergone by a nucleic acid coding sequence controlled by genetic sequences which function in plant cells to achieve production of a desired product, such as a structural gene encoding an RNA molecule, or an RNA molecule being used as a substrate for a reverse transcriptase enzyme or enzyme complex.

Improved or enhanced expression cassette refers to the specific combination and order of genetic elements associated with the insecticidal protein encoding sequence which, when expressed within a plant cell:

gives rise to the surprising average level of that protein expressed in plants, plant tissue, or plant cells;

gives rise to the unexpected number of transformation events expressing a surprisingly higher average level of insecticidal protein;

gives rise to individual plants, plant tissue, or plant cells expressing an unexpectedly high level of the insecticidal protein; and

gives rise to plants expressing unexpected levels of insecticidal protein effective in controlling or combating Coleopteran pests and preventing development of resistance by the Coleopteran pest to the particular insecticidal protein.

Insecticidal polypeptide refers to a polypeptide having insecticidal properties, *e.g.*, a polypeptide which exhibits the properties of inhibiting the growth, development, viability or fecundity of target insect pests.

Operably Linked: Nucleic acid or polynucleotide sequences connected sequentially in linear form, so that the properties of one influence the expression characteristics of the other. A promoter, for example, operably linked to other polynucleotide sequences (which may consist of operator or enhancer sequences, untranslated or translated leader sequences, intron sequences,

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structural gene coding sequences, non-structural genes, transcription and translation termination sequences, and polyadenylation sequences) influences the expression of a coding or noncoding sequence, whether the product is RNA, protein, or other product. Similarly, an intron or an untranslated leader sequence can influence the expression and stability of sequences operably
5 linked to them, and structural or non-structural gene sequences can be influenced by elements operably linked upstream, within, or downstream.

Plant-Expressible Coding Regions: Amino acid coding regions or open reading frames (ORF's) which are expressible *in planta* because they contain typical plant regulatory elements facilitating their expression, and often include changes to the coding sequence such that plant
10 preferred codons are utilized in place of non-preferred codons where heterologous coding regions are contemplated.

Plastid Transit Peptide: Any amino acid sequence useful in targeting a linked amino acid, such as a protein fusion, to a subcellular compartment or organelle such as a plastid or chloroplast.

Polynucleotide sequence: Any DNA or RNA sequence of four or more consecutive
15 nucleotides or ribonucleotides. Generally polynucleotide sequences as disclosed herein comprise at least 50 or more nucleotides or ribonucleotides.

Progeny: "Progeny" includes any offspring or descendant of the transgenic plant, or any subsequent plant which contains the transgene(s) in operable form. Progeny is not limited to one
20 generation, but rather encompasses the transformant's descendants so long as they contain or express the transgene(s). Seeds containing transgenic embryos as well as seeds from the transgenic plants and their offspring or descendants which, after Mendelian segregation continue to contain the transgene(s), are also important parts of the invention.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that
25 provide an expression control element for a preferred polynucleotide sequence and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that preferred sequence.

R₀ is the primary regenerant plant derived from transformation of plant tissue or cells in culture. Subsequent progeny or generations derived from the R₀ are referred to as R₁ (first
30 generation), R₂ (second generation), *etc.*

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Regeneration: The process of growing a plant from a plant cell or group of plant cells (e.g., plant protoplast, embryo, callus, or explant).

Structural Coding Sequence refers to a DNA sequence that encodes a peptide, polypeptide, or protein that is made by a cell following transcription of the structural coding
5 sequence to messenger RNA (mRNA), followed by translation of the mRNA to the desired peptide, polypeptide, or protein product.

Structural gene: A gene or polynucleotide sequence containing the coding sequence of a desired polypeptide that is expressed by transcription and translation to produce the desired polypeptide.

10 **Synthetic gene:** Synthetic genes encoding the *B. thuringiensis* δ -endotoxins of the present invention are those prepared in a manner involving any sort of genetic isolation or manipulation which alters the naturally occurring coding sequence of the δ -endotoxin gene. This includes isolation of the gene from its naturally occurring state, manipulation of the gene as by codon modification (as described herein), or site-specific mutagenesis (as described herein),
15 truncation of the gene or any other manipulative or isolative method. A synthetic gene can also be a polynucleotide sequence which is not known to be naturally occurring but which encodes a useful polypeptide or other product such as a tRNA or an antisense polynucleotide. A non-naturally occurring polynucleotide sequence.

Substantial homology: As this term is used herein, it refers to nucleic acid or
20 polypeptide sequences which are about 86% homologous, to about 90% homologous, to about 95% homologous, to about 99% homologous. More specifically, the inventors envision substantial homologues to be about 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, and 99 percent homologous to the referent nucleic acid sequence of polypeptide.

Terminator: With reference to eukaryotic nuclear gene expression processes, the
25 operable 3' end transcription termination and polyadenylation sequence. With reference to prokaryotic gene expression, and including plastid or chloroplast gene expression, the operable DNA sequence at the 3' end of an open reading frame which, for ORF's expressing protein product, at least one termination codon in frame with the coding sequence of the ORF, which may also be followed by a DNA sequence encoding a transcription termination signal which may
30 cause the translated RNA or mRNA product to form a hairpin or other three dimensional

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structure which may or may not act together with one or more soluble structural proteins to cause transcription to be interrupted.

Transformation: A process of introducing an exogenous polynucleotide sequence (*e.g.*, a vector, or a recombinant or non-recombinant DNA or RNA molecule) into a cell or protoplast in which that exogenous polynucleotide is incorporated into a heritable genetic element or is capable of autonomous replication and thus stably maintained within that cell or protoplast as well as in the progeny of that cell or protoplast.

Transformed cell: A cell which contains a heritable genetic element altered by the introduction of one or more exogenous DNA molecules. A transgenic cell. Exemplary transformed or transgenic cells include plant calli derived from a transformed plant cell and particular cells such as leaf, root, stem, *e.g.*, somatic cells, or reproductive (germ) cells obtained from a transgenic plant.

Transgene: A gene construct, expression cassette, or DNA segment or sequence comprising an ORF which is desired to be expressed in the recipient cell, tissue or organism. This may include an entire plasmid, or other vector, or may simply include the functional coding sequence, region, domain, or segment of the transferred DNA sequence.

Transgenic event: A plant or progeny thereof derived from a plant cell or protoplast manufactured or constructed to contain one or more exogenous DNA molecules inserted into the nuclear or other genome of the plant cell, or introduced and stably maintained within the cytoplasm of a plastid, chloroplast, or mitochondria, which confers some physically detectable phenotype upon the plant or progeny thereof.

Transgenic plant: A plant or progeny thereof which has been genetically modified to contain and express heterologous DNA sequences either as proteins or as nucleic acids. As specifically exemplified herein, a transgenic corn plant is genetically modified to contain and express at least one heterologous DNA sequence operably linked to and under the regulatory control of transcriptional control sequences which function together in plant cells or tissue or in whole plants to achieve expression from a nucleic acid sequence encoding an insecticidal δ -endotoxin protein or an amino acid sequence variant thereof. A transgenic plant may also be referred to as a transformed plant. A transgenic plant also refers to progeny of the initial transgenic plant where those progeny contain and express the heterologous coding sequence

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under the regulatory control of the plant-expressible transcription control sequences described herein.

Vector: A polynucleotide capable of replication in a host cell and/or to which another polynucleotide sequence can be operatively linked so as to bring about replication of the linked
5 sequence. A plasmid is an exemplary vector.

The present invention discloses novel DNA constructs comprising polynucleotide sequences encoding *B. thuringiensis* δ -endotoxins. Methods for the construction and expression of synthetic *B. thuringiensis* genes in plants are well known by those of skill in the art and are described in detail in U. S. Patent 5,500,365. The present invention contemplates the use of
10 Cry3B *B. thuringiensis* genes in the transformation of both monocotyledonous and dicotyledonous plants. To potentiate the expression of these genes, the present invention provides DNA constructs comprising polynucleotide segments encoding plastid targeting peptides positioned upstream of and in frame with the polynucleotide sequences encoding the desired *B. thuringiensis* δ -endotoxins, along with various combinations of untranslated leader
15 sequences, plant functional intron sequences, and transcription termination and polyadenylation sequences.

In one aspect, nucleotide sequence information provided by the invention allows for the preparation of relatively short DNA sequences having the ability to specifically hybridize to gene sequences of the selected polynucleotides disclosed herein. In these aspects, nucleic acid probes
20 of an appropriate length are prepared based on a consideration of selected polypeptide sequences encoding Coleopteran inhibitory Cry3B δ -endotoxin polypeptides, *e.g.*, a sequence such as that shown in SEQID NO:2, SEQID NO:4, SEQID NO:6, SEQID NO:8, SEQID NO:10, and SEQID NO:12. These nucleic acid probes may also be prepared based on a consideration of selected polynucleotide sequences encoding a plastid targeting peptide, such as those shown in SEQID
25 NO:26. The ability of such nucleic acid probes to specifically hybridize to a gene sequence encoding a δ -endotoxin polypeptide or a plastid targeting peptide sequence lends to them particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence
30 of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of a crystal protein gene from *B. thuringiensis* using

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thermal amplification technology. The process may also be used to detect, amplify or mutate a defined segment of the polynucleotide encoding a plastid targeting peptide. Segments of genes related to the polynucleotides encoding the δ -endotoxin polypeptides and plastid targeting peptides of the present invention may also be amplified by using such primers and thermal
5 amplification methods.

To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes a polynucleotide sequences at least about 14 to 30 or so nucleotides in length complimentary to a nucleotide sequence encoding a crystal protein, or polynucleotide sequences at least about 14 to 30 or so
10 nucleotides in length complimentary to a nucleotide sequence encoding a plastid targeting peptide.

A size of at least 14 nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over segments greater than 14 bases in length are generally preferred.
15 In order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained, one will generally prefer to design nucleic acid molecules having gene-complementary sequences of 14 to 20 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the
20 PCR™ technology of U. S. Patents 4,683,195, and 4,683,202, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction sites.

The present invention also contemplates an expression vector comprising a polynucleotide of the present invention. Thus, in one embodiment an expression vector is an
25 isolated and purified DNA molecule comprising a promoter operatively linked to a coding region that encodes a polypeptide of the present invention, which coding region is operatively linked to a transcription-terminating region, whereby the promoter drives the transcription of the coding region. The coding region may include a segment encoding a *B. thuringiensis* δ -endotoxin and a segment encoding a plastid target peptide. The DNA molecule comprising the expression vector
30 may also contain a functional intron. As used herein, the terms "operatively linked" or "operably linked" mean that a promoter is connected to a coding region in such a way that the transcription

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of that coding region is controlled and regulated by that promoter. Means for operatively linking a promoter to a coding region to regulate both upstream and downstream are well known in the art.

Preferred plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, *e.g.*, by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and Eur. Pat Appl. No. EP 0120516.

Promoters that function in bacteria are well known in the art. Exemplary and preferred promoters for the *B. thuringiensis* crystal proteins include the *sigA*, *sigE*, and *sigK* gene promoters. Alternatively, native, mutagenized, heterologous, or recombinant promoters derived from *Bacillus thuringiensis* δ -endotoxin protein coding sequences can be used.

Where an expression vector of the present invention is to be used to transform a plant, a promoter is selected that has the ability to drive expression in that particular species of plant. Promoters that function in different plant species are also well known in the art. Promoters useful in expression of polypeptide coding sequences in plants are those which are inducible, viral, synthetic, or constitutive as described (Poszkowski *et al.*, 1989; Odell *et al.*, 1985), and/or temporally regulated, spatially regulated, and spatio-temporally regulated (Chau *et al.*, 1989). Preferred promoters include the enhanced CaMV35S promoters, and the FMV35S promoter. Other promoters include the POX promoter, the ScbDNA virus early promoter, and the yellow mottle virus promoter.

In accordance with the present invention, expression vectors designed to specifically potentiate the expression of the polypeptide in the transformed plant may include certain regions encoding plastid targeting peptides (PTP). These regions allow for the cellular processes involved in transcription, translation and expression of the encoded protein to be fully exploited when associated with certain *B. thuringiensis* δ -endotoxins. Such plastid targeting peptides function in a variety of ways, such as for example, by transferring the expressed protein to the cell structure in which it most effectively operates, or by transferring the expressed protein to areas of the cell in which cellular processes necessary for expression are concentrated.

In the case of Cry3B, elevated expression is critical in obtaining transgenic corn with CRW control since the LC_{50} of Cry3B against CRW is significantly higher than the LC_{50} of the *B. thuringiensis* toxins currently used to control pests such as Colorado Potato Beetle in potato (Cry3A) or European Corn Borer in corn (Cry1Ab).

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Increased expression is also especially valuable in that it provides additional protection against development of resistance *via* a high dose strategy (McGaughey and Whalon, 1993; Roush, 1994). High level expression is even further desirable as it provides sustained insect protection in instances where insecticidal gene expression decreases due to environmental
5 conditions. Additionally and unexpectedly, corn plants transformed with vectors expressing Coleopteran inhibitory Cry3B or variant proteins exhibited normal growth and development.

An example of a plastid or chloroplast targeting peptide (CTP) is a chloroplast targeting peptide. Chloroplast targeting peptides have been found particularly useful in the glyphosate resistant selectable marker system. In this system, plants transformed to express a protein
10 conferring glyphosate resistance are transformed with a PTP that targets the peptide to the cell's chloroplasts. Glyphosate inhibits the shikimic acid pathway which leads to the biosynthesis of aromatic compounds including amino acids and vitamins. Specifically, glyphosate inhibits the conversion of phosphoenolpyruvic acid and 3-phosphoshikimic acid to 5-enolpyruvyl-3-phosphoshikimic acid by inhibiting the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase
15 (EPSP synthase or EPSPS). Supplemental EPSPS, conferred *via* insertion of a transgene encoding this enzyme, allows the cell to resist the effects of the glyphosate. Thus, as the herbicide glyphosate functions to kill the cell by interrupting aromatic amino acid biosynthesis, particularly in the cell's chloroplast, the CTP allows increased resistance to the herbicide by concentrating what glyphosate resistance enzyme the cell expresses in the chloroplast, *i.e.* in the
20 target organelle of the cell. Exemplary herbicide resistance enzymes include EPSPS as noted above, glyphosate oxido-reductase (GOX) and the aro-A gene (U.S. Patent No. 4,535,060).

CTP's can target proteins to chloroplasts and other plastids. For example, the target organelle may be the amyloplast. Preferred CTP's of the present invention include those targeting both chloroplasts as well as other plastids. Specific examples of preferred CTP's include the
25 maize RUBISCO SSU protein CTP, and functionally related peptides. An exemplary CTP polypeptide is shown in SEQ ID NO:26. A polynucleotide sequence encoding for this CTP polypeptide is shown in SEQ ID NO:25.

The expression of a gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from the coding strand of the DNA by an RNA
30 polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to

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as the "promoter". The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA. The particular promoter selected should be capable of causing sufficient expression of the enzyme coding sequence to
5 result in the production of an effective insecticidal amount of the *B. thuringiensis* protein.

The 3' non-translated region of the chimeric plant genes of the present invention also contains a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3' end of the RNA. Examples of preferred 3' regions are (1) the 3' transcribed, non-translated regions containing the polyadenylation signal of *Agrobacterium* tumor-inducing
10 (Ti) plasmid genes, such as the nopaline synthase (NOS) gene and (2) the 3' ends of plant genes such as the pea ssRUBISCO E9 gene (Fischhoff *et al.*, 1987).

A promoter is selected for its ability to direct the transformed plant cell's or transgenic plant's transcriptional activity to the coding region, to ensure sufficient expression of the enzyme coding sequence to result in the production of insecticidal amounts of the *B. thuringiensis*
15 protein. Structural genes can be driven by a variety of promoters in plant tissues. Promoters can be near-constitutive (*i.e.* they drive transcription of the transgene in all tissue), such as the CaMV35S promoter, or tissue-specific or developmentally specific promoters affecting dicots or monocots. Where the promoter is a near-constitutive promoter such as CaMV35S or FMV35S, increases in polypeptide expression are found in a variety of transformed plant tissues and most
20 plant organs (*e.g.*, callus, leaf, seed and root). Enhanced or duplicate versions of the CaMV35S and FMV35S promoters are particularly useful in the practice of this invention (Kay *et al.*, 1987; Rogers, U. S. Patent 5,378,619). Tandemly duplicated enhancer sequences have been demonstrated to be of particular significance, for example, as described in Neuhaus *et al.* (Tissue-specific expression from promoter AS-1 in transgenic tobacco. *Plant Cell* 6: 827-834;
25 1994).

Those skilled in the art will recognize that there are a number of promoters which are active in plant cells, and have been described in the literature. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on tumor-inducing plasmids of
30 *A. tumefaciens*), the cauliflower mosaic virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose 1,5-bisphosphate carboxylase

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(ssRUBISCO, a very abundant plant polypeptide), the rice *Act1* promoter, POX promoter, yellow mottle virus promoter, ScBV virus early promoter, the Figwort Mosaic Virus (FMV) 35S promoter, and the AS4 35S promoter (root enhanced expression from 35S promoter linked to multiple tandem as-1 sequences as in Neuhaus et al.). All of these promoters have been used to
5 create various types of DNA constructs which have been expressed in plants (see *e.g.*, McElroy *et al.*, 1990, U. S. Patent 5,463,175).

In addition, it may also be preferred to bring about expression of the *B. thuringiensis* δ -endotoxin in specific tissues of the plant by using plant integrating vectors containing a tissue-specific promoter. Specific target tissues may include the leaf, stem, root, tuber, seed, fruit, *etc.*,
10 and the promoter chosen should have the desired tissue and developmental specificity. Therefore, promoter function should be optimized by selecting a promoter with the desired tissue expression capabilities and approximate promoter strength and selecting a transformant which produces the desired insecticidal activity in the target tissues. This selection approach from the pool of transformants is routinely employed in expression of heterologous structural genes in plants
15 since there is variation between transformants containing the same heterologous gene due to the site of gene insertion within the plant genome (commonly referred to as "position effect"). In addition to promoters which are known to cause transcription (constitutive or tissue-specific) of DNA in plant cells, other promoters may be identified for use in the current invention by screening a plant cDNA library for genes which are selectively or preferably expressed in the
20 target tissues and then determine the promoter regions.

An exemplary tissue-specific promoter is the lectin promoter, which is specific for seed tissue. The lectin protein in soybean seeds is encoded by a single gene (*Le1*) that is only expressed during seed maturation and accounts for about 2 to about 5% of total seed mRNA. The lectin gene and seed-specific promoter have been fully characterized and used to direct seed
25 specific expression in transgenic tobacco plants (Vodkin *et al.*, 1983; Lindstrom *et al.*, 1990). An expression vector containing a coding region that encodes a polypeptide of interest can be engineered to be under control of the lectin promoter and that vector may be introduced into plants using, for example, a protoplast transformation method (Dhir *et al.*, 1991). The expression of the polypeptide would then be directed specifically to the seeds of the transgenic
30 plant.

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A transgenic plant of the present invention produced from a plant cell transformed with a tissue specific promoter can be crossed with a second transgenic plant developed from a plant cell transformed with a different tissue specific promoter to produce a hybrid transgenic plant that shows the effects of transformation in more than one specific tissue.

5 Other exemplary tissue-specific promoters are corn sucrose synthetase 1 (Yang *et al.*, 1990), corn alcohol dehydrogenase 1 (Vogel *et al.*, 1989), corn light harvesting complex (Simpson, 1986), corn heat shock protein (Odell *et al.*, 1985), pea small subunit RuBP carboxylase (Poulsen *et al.*, 1986; Cashmore *et al.*, 1983), Ti plasmid mannopine synthase (McBride and Summerfelt, 1989), Ti plasmid nopaline synthase (Langridge *et al.*, 1989), petunia
10 chalcone isomerase (Van Tunen *et al.*, 1988), bean glycine rich protein 1 (Keller *et al.*, 1989), CaMV 35s transcript (Odell *et al.*, 1985) and Potato patatin (Wenzler *et al.*, 1989). Preferred promoters are the cauliflower mosaic virus (CaMV 35S) promoter and the S-E9 small subunit RuBP carboxylase promoter.

The promoters used in the DNA constructs of the present invention may be modified, if
15 desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represses the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein. For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, *e.g.*, promoters derived
20 by means of ligation with operator regions, random or controlled mutagenesis, *etc.* Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression. Examples of such enhancer sequences have been reported by Kay *et al.* (1987) and Neuhaus *et al.* (1994).

The RNA produced by a DNA construct of the present invention also contains a 5' non-
25 translated leader sequence. This sequence can be derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5' non-translated regions can also be obtained from viral RNAs, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs wherein the non-translated region is derived from the 5' non-translated sequence that accompanies the
30 promoter sequence. As shown below, a plant gene leader sequence which is useful in the present

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invention is the petunia heat shock protein 70 (hsp70) leader (Winter *et al.*, 1988), the wheat CAB leader, or the wheat PER leader.

An exemplary embodiment of the invention involves the plastid targeting of the *B. thuringiensis* sequence. Such plastid targeting sequences have been isolated from numerous nuclear encoded plant genes and have been shown to direct importation of cytoplasmically synthesized proteins into plastids (reviewed in Keegstra and Olsen, 1989). A variety of plastid targeting sequences, well known in the art, including but not limited to ADPGPP, EPSP synthase, or ssRUBISCO, may be utilized in practicing this invention. In alternative embodiments preferred, plastidic targeting sequences (peptide and nucleic acid) for monocotyledonous crops may consist of a genomic fragment coding containing an intronic sequence as well as a duplicated proteolytic cleavage site in the encoded plastidic targeting sequences.

The most preferred CTP encoding nucleic acid sequence, referred to herein as zmSSU CTP (SEQ ID NO:25), consisting of a genomic fragment containing an intronic sequence as well as a duplicated proteolytic cleavage site in the encoded plastidic targeting sequences, was derived from plastidic targeting sequence zmS1 (Russell *et al.*, 1993). Direct translational fusions of zmSSU CTP peptide sequence (SEQ ID NO:26) to the amino terminus of the sequence has been shown to be useful in obtaining elevated levels of the polypeptide in transgenic maize. In-frame fusions of the zmSSU CTP nucleic acid sequence (SEQ ID NO:25) to a *cry3b* gene (SEQ ID NO:1) or gene variant can be effected by ligation of an *NcoI* site engineered into the 3' (C-terminal encoding) end of the zmSSU CTP sequence to a 5' *NcoI* site engineered into the N-terminal encoding end of the *cry3B* or variant coding sequence.

The preferred CTP sequence for dicotyledonous crops consists of a genomic coding fragment containing the chloroplast targeting peptide sequence from the EPSP synthase gene of *Arabidopsis thaliana* in which the transit peptide cleavage site of the pea ssRUBISCO CTP replaces the native EPSP synthase CTP cleavage site (Klee *et al.*, 1987).

As noted above, the 3' non-translated region of the chimeric plant genes of the present invention contains a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3' end of the RNA. Examples of preferred 3' regions are (1) the transcribed, non-translated regions containing the polyadenylate signal of *Agrobacterium* tumor-

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inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene and (2) plant genes such as the pea ssRUBISCO E9 gene (Fischhoff *et al.*, 1987).

For optimized expression in monocotyledonous plants, an intron may also be included in the DNA expression construct. Such an intron is typically placed near the 5'-end of the mRNA in an untranslated sequence. This intron could be obtained from, but not limited to, a set of introns consisting of the maize Heat Shock Protein (HSP) 70 intron (U. S. Patent 5,424,412; 1995), the rice *Act1* intron (McElroy *et al.*, 1990), the Adh intron 1 (Callis *et al.*, 1987), or the sucrose synthase intron (Vasil *et al.*, 1989). As shown herein, the maize HSP70 intron (SEQID NO:33) and the rice actin intron (SEQID NO:32) are particularly useful in the present invention.

RNA polymerase transcribes through a coding DNA sequence to a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA).

Constructs will typically include the gene of interest along with a 3' end DNA sequence that acts as a signal to terminate transcription and allow for the poly-adenylation of the resultant mRNA. The most preferred 3' elements are contemplated to be those from the nopaline synthase gene of *A. tumefaciens* (*nos* 3'end) (Bevan *et al.*, 1983), the terminator for the T7 transcript from the octopine synthase gene of *A. tumefaciens*, and the 3' end of the protease inhibitor i or ii genes from potato or tomato. Regulatory elements such as TMV Ω element (Gallie, *et al.*, 1989), may further be included where desired.

Another type of element which can regulate gene expression is the DNA sequence between the transcription initiation site and the start of the coding sequence, termed the untranslated leader sequence. The leader sequence can influence gene expression. Compilations of leader sequences have been made to predict optimum or sub-optimum sequences and generate "consensus" and preferred leader sequences (Joshi, 1987). Preferred leader sequences are contemplated to include those which comprise sequences predicted to direct optimum expression of the linked structural gene, *i.e.* to include a preferred consensus leader sequence which may increase or maintain mRNA stability and prevent inappropriate initiation of translation. The

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choice of such sequences will be known to those of skill in the art in light of the present disclosure. Sequences that are derived from genes that are highly expressed in plants, and in maize in particular, will be most preferred. One particularly preferred leader may be the wheat CAB leader (SEQID NO:31).

5 Transcription enhancers or duplications of enhancers could be used to increase expression. These enhancers often are found 5' to the start of transcription in a promoter that functions in eukaryotic cells, but can often be inserted in the forward or reverse orientation 5' or 3' to the coding sequence. Examples of enhancers include elements from the CaMV 35S promoter, octopine synthase genes (Ellis *et al.*, 1987), the rice actin gene, and promoter from
10 non-plant eukaryotes (*e.g.*, yeast; Ma *et al.*, 1988).

The choice of which expression vector and ultimately to which promoter a polypeptide coding region is operatively linked depends directly on the functional properties desired, *e.g.*, the location and timing of protein expression, and the host cell to be transformed. These are well known limitations inherent in the art of constructing recombinant DNA molecules. However, a
15 vector useful in practicing the present invention is capable of directing the expression of the polypeptide coding region to which it is operatively linked.

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *A. tumefaciens* described (Rogers *et al.*, 1987). However, several other plant integrating vector systems are known to
20 function in plants including pCaMVCN transfer control vector described (Fromm *et al.*, 1985). pCaMVCN (available from Pharmacia, Piscataway, NJ) includes the CaMV35S promoter.

In preferred embodiments, the vector used to express the polypeptide includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin resistance;
25 *i.e.* the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II (*nptII*) and nopaline synthase 3' non-translated region described (Rogers *et al.*, 1988).

Means for preparing expression vectors are well known in the art. Expression (transformation) vectors used to transform plants and methods of making those vectors are
30 described in U. S. Patents 4,971,908, 4,940,835, 4,769,061 and 4,757,011. Those vectors can be modified to include a coding sequence in accordance with the present invention.

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A coding region that encodes a polypeptide having the ability to confer insecticidal activity to a cell is preferably a polynucleotide encoding a *B. thuringiensis* δ -endotoxin or a functional equivalent of such a polynucleotide. In accordance with such embodiments, a coding region comprising the DNA sequences of SEQID NO:1, SEQID NO:3, SEQID NO:5, SEQID NO:7, SEQID NO:9, and SEQID NO:11 are also preferred.

Specific *B. thuringiensis* δ -endotoxin polypeptide-encoding ORF's contained within expression cassettes that have been shown to express the *B. thuringiensis* δ -endotoxins at high levels in transformed plants. Preferred cassettes include those contained in plasmids pMON33709, pMON33710, pMON33722, pMON33723, pMON25096, pMON25097, pMON33741, and pMON33748. The expression cassettes in these plasmids are respectively encoded for by the sequences shown in SEQID NO:13, SEQID NO:15, SEQID NO:36, SEQID NO:38, SEQID NO:17, SEQID NO:19, SEQID NO:21, and SEQID NO:23. More preferably, plants may be successfully transformed with any expression cassettes comprising the nucleotide sequences of nucleotide 14 to 3431 of SEQID NO:36, 14 to 3025 of SEQID NO:38, 14 to 3431 of SEQID NO:17, 14 to 3020 of SEQID NO:19, 14 to 3020 of SEQID NO:21, or 25 to 3450 of SEQID NO:23 (pMON33722, pMON33723, pMON25096, pMON25097, pMON33741, and pMON33748). Most preferably, plants may be successfully transformed with any expression cassettes comprising the nucleotide sequences of nucleotide 14 to 3431 of SEQID NO:17, 14 to 3020 of SEQID NO:19, 14 to 3020 of SEQID NO:21, or 25 to 3450 of SEQID NO:23 (pMON25096, pMON25097, pMON33741, and pMON33748).

The work described herein has identified methods of potentiating *in planta* expression of *B. thuringiensis* δ -endotoxins, which confer resistance to insect pathogens when incorporated into the genome of susceptible plants. U. S. Patent 5,500,365 describes a method for synthesizing plant genes to optimize the expression level of the protein for which the synthesized gene encodes. This method relates to the modification of the structural gene sequences of the exogenous transgene, to make them more "plant-like" and therefore more likely to be translated and expressed by the plant. A similar method for enhanced expression of transgenes in monocotyledonous plants is disclosed in U. S. Patent 5,689,052. Agronomic, horticultural, ornamental, and other economically or commercially useful plants can be made in accordance

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with the methods described herein, to express *B. thuringiensis* δ -endotoxins at levels high enough to confer resistance to insect pathogens.

Such plants may co-express the *B. thuringiensis* δ -endotoxin polypeptide along with other antifungal, antibacterial, or antiviral pathogenesis-related peptides, polypeptides, or proteins; insecticidal proteins; proteins conferring herbicide resistance; and proteins involved in improving the quality of plant products or agronomic performance of plants. Simultaneous co-expression of multiple proteins in plants is advantageous in that it exploits more than one mode of action to control plant pathogenic damage. This can minimize the possibility of developing resistant pathogen strains, broaden the scope of resistance, and potentially result in a synergistic insecticidal effect, thereby enhancing plants ability to resist insect infestation (WO 92/17591).

Ultimately, the most desirable DNA segments for introduction into a monocot genome may be homologous genes or gene families which encode a desired trait (for example, increased yield), and which are introduced under the control of novel promoters or enhancers, *etc.*, or perhaps even homologous or tissue specific (*e.g.*, root-collar / sheath-, whorl-, stalk-, earshank-, kernel- or leaf-specific) promoters or control elements. Indeed, it is envisioned that a particular use of the present invention may be the production of transformants comprising a transgene which is targeted in a tissue-specific manner. For example, insect resistant genes may be expressed specifically in the whorl and collar/sheath tissues which are targets for the first and second broods, respectively, of ECB. Likewise, it is desirable that genes encoding proteins with particular activity against rootworm be preferentially expressed in root tissues.

Vectors for use in tissue-specific targeting of gene expression in transgenic plants typically will include tissue-specific promoters and also may include other tissue-specific control elements such as enhancer sequences. Promoters which direct specific or enhanced expression in certain plant tissues will be known to those of skill in the art in light of the present disclosure.

It also is contemplated that tissue specific expression may be functionally accomplished by introducing a constitutively expressed gene (all tissues) in combination with an antisense gene that is expressed only in those tissues where the gene product is not desired. For example, a gene coding for the crystal toxin protein from *B. thuringiensis* may be introduced such that it is expressed in all tissues using the 35S promoter from Cauliflower Mosaic Virus. Alternatively, a rice actin promoter or a histone promoter from a dicot or monocot species also could be used for constitutive expression of a gene. Furthermore, it is contemplated that promoters combining

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elements from more than one promoter may be useful. For example, U. S. Patent 5,491,288 discloses combining a Cauliflower Mosaic Virus promoter with a histone promoter. Therefore, expression of an antisense transcript of a Bt δ -endotoxin gene in a maize kernel, using for example a zein promoter, would prevent accumulation of the δ -endotoxin in seed. Hence the protein encoded by the introduced gene would be present in all tissues except the kernel. It is specifically contemplated by the inventors that a similar strategy could be used with the instant invention to direct expression of a screenable or selectable marker in seed tissue.

Alternatively, one may wish to obtain novel tissue-specific promoter sequences for use in accordance with the present invention. To achieve this, one may first isolate cDNA clones from the tissue concerned and identify those clones which are expressed specifically in that tissue, for example, using Northern blotting. Ideally, one would like to identify a gene that is not present in a high copy number, but which gene product is relatively abundant in specific tissues. The promoter and control elements of corresponding genomic clones may thus be localized using the techniques of molecular biology known to those of skill in the art.

It is contemplated that expression of some genes in transgenic plants will be desired only under specified conditions. For example, it is proposed that expression of certain genes that confer resistance to environmentally stress factors such as drought will be desired only under actual stress conditions. It further is contemplated that expression of such genes throughout a plants development may have detrimental effects. It is known that a large number of genes exist that respond to the environment. For example, expression of some genes such as *rbcS*, encoding the small subunit of ribulose biphosphate carboxylase, is regulated by light as mediated through phytochrome. Other genes are induced by secondary stimuli. For example, synthesis of abscisic acid (ABA) is induced by certain environmental factors, including but not limited to water stress. A number of genes have been shown to be induced by ABA (Skriver and Mundy, 1990). It also is expected that expression of genes conferring resistance to insect predation would be desired only under conditions of actual insect infestation. Therefore, for some desired traits, inducible expression of genes in transgenic plants will be desired.

It is proposed that, in some embodiments of the present invention, expression of a gene in a transgenic plant will be desired only in a certain time period during the development of the plant. Developmental timing frequently is correlated with tissue specific gene expression. For

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example expression of zein storage proteins is initiated in the endosperm about 15 days after pollination.

It is contemplated that the method described in this invention could be used to obtain substantially improved expression of a number of novel *B. thuringiensis* endotoxins isolated as described below. Identification of new *Bacillus thuringiensis* strains encoding crystalline endotoxins with insecticidal activity has been described previously (Donovan *et al.*, 1992). Isolation of the *B. thuringiensis* endotoxin, followed by amino terminal amino acid sequencing, back-translation of the amino acid sequence to design an oligonucleotide probe or use of a related *B. thuringiensis* gene as a probe, followed by cloning of the gene encoding the endotoxin by hybridization are familiar to those skilled in the art and have been described (see *e.g.*, Donovan *et al.*, 1992, U. S. Patent 5,264,364). Cry3Bb *Bacillus thuringiensis* δ -endotoxins with improved Coleopteran inhibitory activity can be achieved using the methods described in English *et al.* (WO99/31248).

A plant transformed with an expression vector of the present invention is also contemplated. A transgenic plant derived from such a transformed or transgenic cell is also contemplated. Those skilled in the art will recognize that a chimeric plant gene containing a structural coding sequence of the present invention can be inserted into the genome of a plant by methods well known in the art. Such methods for DNA transformation of plant cells include *Agrobacterium*-mediated plant transformation, the use of liposomes, transformation using viruses or pollen, electroporation, protoplast transformation, gene transfer into pollen, injection or vacuum infiltration (Bechtold *et al.*, Meth. Mo. Biol., 82:259-266; 1998) into reproductive organs, injection into immature embryos and particle bombardment. Each of these methods has distinct advantages and disadvantages. Thus, one particular method of introducing genes into a particular plant strain may not necessarily be the most effective for another plant strain, but it is well known which methods are useful for a particular plant strain.

Technology for introduction of DNA into cells is well-known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, 1973); (2) physical methods such as microinjection (Capecchi, 1980), electroporation (Wong and Neumann, 1982; Fromm *et al.*, 1985) and the gene gun (Johnston and Tang, 1994; Fynan *et al.*, 1993); (3) viral vectors (Clapp, 1993; Lu *et al.*, 1993; Eglitis and

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Anderson, 1988a; 1988b); and (4) receptor-mediated mechanisms (Curiel *et al.*, 1991; 1992; Wagner *et al.*, 1992).

An advantageous method for delivering transforming DNA segments to plant cells is microprojectile bombardment. In this method, particles may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like. Using these particles, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al.*, 1987; Klein *et al.*, 1988; Kawata *et al.*, 1988). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

An advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly stably transforming plant cells, is that neither the isolation of protoplasts (Cristou *et al.*, 1988) nor the susceptibility to *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into plant cells by acceleration is a Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with the plant cultured cells in suspension. The screen disperses the particles so that they are not delivered to the recipient cells in large aggregates. It is believed that a screen intervening between the projectile apparatus and the cells to be bombarded reduces the size of projectiles aggregate and may contribute to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large.

For the bombardment, cells in suspension are preferably concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the macroprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from 1 to 10 and average 1 to 3.

In bombardment transformation, one may optimize the prebombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in

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this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment, and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature plant embryos.

Accordingly, it is contemplated that one may desire to adjust various of the bombardment parameters in small scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance, and helium pressure. One may also minimize the trauma reduction factors (TRFs) by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

The methods of particle-mediated transformation is well-known to those of skill in the art. U. S. Patent 5,015,580 describes the transformation of soybeans using such a technique.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described (Fraley *et al.*, 1985; Rogers *et al.*, 1987). The genetic engineering of cotton plants using *Agrobacterium*-mediated transfer is described in U. S. Patent 5,004,863; like transformation of lettuce plants is described in U. S. Patent 5,349,124; and the *Agrobacterium*-mediated transformation of soybean is described in U. S. Patent 5,416,011. Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, 1986; Jorgensen *et al.*, 1987).

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Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, 1985). Moreover, recent technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate
5 construction of vectors capable of expressing various polypeptide coding genes. The vectors described (Rogers *et al.*, 1987), have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant varieties where *Agrobacterium*-mediated
10 transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

Agrobacterium-mediated transformation of leaf disks and other tissues such as cotyledons and hypocotyls appears to be limited to plants that *Agrobacterium* naturally infects. *Agrobacterium*-mediated transformation is most efficient in dicotyledonous plants. Few
15 monocots appear to be natural hosts for *Agrobacterium*, although transgenic plants have been produced in asparagus using *Agrobacterium* vectors as described (Bytebier *et al.*, 1987). Other monocots recently have also been transformed with *Agrobacterium*. Included in this group are corn (Ishida *et al.*) and rice (Cheng *et al.*).

A transgenic plant formed using *Agrobacterium* transformation methods typically
20 contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. However, inasmuch as use of the word "heterozygous" usually implies the presence of a complementary gene at the same locus of the second chromosome of a pair of chromosomes, and there is no such gene in a plant containing one added gene as here, it is believed that a more accurate name for such a plant is an independent segregant, because the
25 added, exogenous gene segregates independently during mitosis and meiosis.

An independent segregant may be preferred when the plant is commercialized as a hybrid, such as corn. In this case, an independent segregant containing the gene is crossed with another plant, to form a hybrid plant that is heterozygous for the gene of interest.

An alternate preference is for a transgenic plant that is homozygous for the added
30 structural gene; *i.e.* a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by

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sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for gene of interest activity and mendelian inheritance indicating homozygosity relative to a control (native, non-transgenic) or an independent segregant transgenic plant.

5 Two different transgenic plants can be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

10 Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see *e.g.*, Potrykus *et al.*, 1985; Lorz *et al.*, 1985; Fromm *et al.*, 1985; Uchimiya *et al.*, 1986; Callis *et al.*, 1987; Marcotte *et al.*, 1988). Application of these systems to different plant germplasm depends upon the ability to regenerate that particular plant variety from
15 protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described (see, *e.g.*, Fujimura *et al.*, 1985; Toriyama *et al.*, 1986; Yamada *et al.*, 1986; Abdullah *et al.*, 1986). To transform plant germplasm that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil,
20 1988). DNA can also be introduced into plants by direct DNA transfer into pollen as described (Zhou *et al.*, 1983; Hess, 1987). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described (Pena *et al.*, 1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of desiccated embryos as described (Neuhaus *et al.*, 1987; Benbrook *et al.*, 1986).

25 Unmodified bacterial genes are often poorly expressed in transgenic plant cells. Several reports have disclosed methods for improving expression of recombinant genes in plants (Murray *et al.*, 1989; Diehn *et al.*, 1996; Iannacone *et al.*, 1997; Rouwendal *et al.*, 1997; Futterer *et al.*, 1997; and Futterer and Hohn, 1996). These reports disclose various methods for engineering coding sequences to represent sequences which are more efficiently translated based
30 on plant codon frequency tables, improvements in codon third base position bias, using recombinant sequences which avoid suspect polyadenylation or A/T rich domains or intron

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splicing consensus sequences. While these methods for synthetic gene construction are notable, synthetic genes of the present invention were prepared according to the method of Brown et al. (US Pat. No. 5,689,052; 1997). Thus, the present invention provides a method for preparing synthetic plant genes express *in planta* a desired protein product at levels significantly higher than the wild-type genes. Briefly, according to Brown et al., the frequency of rare and semi-rare monocotyledonous codons in a polynucleotide sequence encoding a desired protein are reduced and replaced with more preferred monocotyledonous codons. Enhanced accumulation of a desired polypeptide encoded by a modified polynucleotide sequence in a monocotyledonous plant is the result of increasing the frequency of preferred codons by analyzing the coding sequence in successive six nucleotide fragments and altering the sequence based on the frequency of appearance of the six-mers as to the frequency of appearance of the rarest 284, 484, and 664 six-mers in monocotyledonous plants. Furthermore, Brown et al. disclose the enhanced expression of a recombinant gene by applying the method for reducing the frequency of rare codons with methods for reducing the occurrence of polyadenylation signals and intron splice sites in the nucleotide sequence, removing self-complementary sequences in the nucleotide sequence and replacing such sequences with nonself-complementary nucleotides while maintaining a structural gene encoding the polypeptide, and reducing the frequency of occurrence of 5'-CG-3' dinucleotide pairs in the nucleotide sequence. These steps are performed sequentially and have a cumulative effect resulting in a nucleotide sequence containing a preferential utilization of the more-preferred monocotyledonous codons for monocotyledonous plants for a majority of the amino acids present in the desired polypeptide.

Thus, the amount of a gene coding for a polypeptide of interest (*i.e.* a bacterial crystal protein or δ -endotoxin polypeptide or such δ -endotoxin linked to a plastid targeting peptide) can be increased in plants by transforming those plants using transformation methods such as those disclosed herein.

After effecting delivery of exogenous DNA to recipient cells, the next step to obtain a transgenic plant generally concern identifying the transformed cells for further culturing and plant regeneration. As mentioned herein, in order to improve the ability to identify transformants, it is preferable to employ a selectable or screenable marker gene as, or in addition to, the expressible gene of interest. In this case, one would then generally assay the potentially

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transformed cell population by exposing the cells to a selective agent or agents, or one would screen the cells for the desired marker gene trait.

An exemplary embodiment of methods for identifying transformed cells involves exposing the transformed cultures to a selective agent, such as a metabolic inhibitor, an

5 antibiotic, herbicide or the like. Cells which have been transformed and have stably integrated a marker gene conferring resistance to the selective agent used, will grow and divide in culture.

Sensitive cells will not be amenable to further culturing. One example of a preferred marker gene encoding an EPSPS synthase which is resistant to glyphosate inhibition. When this gene is used as a selectable marker, the putatively transformed cell culture is treated with glyphosate.

10 Upon treatment, transgenic cells will be available for further culturing while sensitive, or non-transformed cells, will not. This method is described in detail in U. S. Patent 5,569,834.

Another example of a preferred selectable marker system is the nptII system by which resistance to the antibiotics kanamycin, neomycin, and paromomycin or related antibiotics is conferred, as described in U. S. Patent 5,569,834. Again, after transformation with this system transformed

15 cells containing a plant expressible nptII gene will be available for further culturing upon treatment with kanamycin or related antibiotic, while non-transformed cells will not. Use of this type of a selectable marker system is described in Brown et al. (U S Patent No. 5,424,412).

Another screenable marker which may be used is the gene coding for green fluorescent protein.

All contemplated assays are nondestructive and transformed cells may be cultured further

20 following identification.

It is further contemplated that combinations of screenable and selectable markers will be useful for identification of transformed cells. In some cell or tissue types a selection agent, such as glyphosate or kanamycin, may either not provide enough killing activity to clearly recognize transformed cells or may cause substantial nonselective inhibition of transformants and non-

25 transformants alike, thus causing the selection technique to not be effective. It is proposed that selection with a growth inhibiting compound, such as glyphosate at concentrations below those that cause 100% inhibition followed by screening of growing tissue for expression of a

screenable marker gene such as kanamycin would allow one to recover transformants from cell or tissue types that are not amenable to selection alone. It is proposed that combinations of

30 selection and screening may enable one to identify transformants in a wider variety of cell and tissue types.

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The development or regeneration of plants from either single plant protoplasts or various explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted
5 plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch *et al.*, 1985). In this
10 procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described (Fraley *et al.*, 1983). In particular, U. S. Patent 5,349,124 details the creation of genetically transformed lettuce cells and plants resulting therefrom which express hybrid crystal proteins conferring insecticidal activity against *Lepidopteran* larvae to such plants. This procedure typically
15 produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

A transgenic plant of this invention thus has an increased amount of a coding region encoding a *B. thuringiensis* δ -endotoxin polypeptide or variant thereof or may encode such a δ -endotoxin linked to a plastid targeting peptide. A preferred transgenic plant is an independent segregant and can transmit that gene and its activity to its progeny. A more preferred transgenic plant is homozygous for that gene, and transmits that gene to all of its offspring on sexual
25 mating. Seed from a transgenic plant may be grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for increased expression of the transgene encoding the δ -endotoxin.

To identify a transgenic plant expressing high levels of the δ -endotoxin of interest, it is
30 necessary to screen the herbicide or antibiotic resistant transgenic, regenerated plants (R_0 generation) for insecticidal activity and/or expression of the gene of interest. This can be

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accomplished by various methods well known to those skilled in the art, including but not limited to: 1) obtaining small tissue samples from the transgenic R₀ plant and directly assaying the tissue for activity against susceptible insects in parallel with tissue derived from a non-expressing, negative control plant. For example, R₀ transgenic corn plants expressing *B.*

5 *thuringiensis* endotoxins such as Cry3B can be identified by assaying leaf tissue or root tissue derived from such plants for activity against CRW; 2) analysis of protein extracts by enzyme linked immunoassays (ELISAs) specific for the gene of interest (Cry3B); or 3) reverse transcriptase thermal amplification to identify events expressing the gene of interest.

The genes and δ -endotoxins according to the subject invention include not only the full
10 length sequences disclosed herein but also fragments of these sequences, or fusion proteins, which retain the characteristic insecticidal activity of the sequences specifically exemplified herein.

It should be apparent to a person of skill in the art that insecticidal δ -endotoxins can be identified and obtained through several means. The specific genes, or portions thereof, may be
15 obtained from a culture depository, or constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal31* or site-directed mutagenesis can be used to systematically cut off
20 nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these δ -endotoxins.

Equivalent δ -endotoxins and/or genes encoding these δ -endotoxins can also be isolated from *Bacillus* strains and/or DNA libraries using the teachings provided herein. For example,
25 antibodies to the δ -endotoxins disclosed and claimed herein can be used to identify and isolate other δ -endotoxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the δ -endotoxins which are most constant and most distinct from other *B. thuringiensis* δ -endotoxins. These antibodies can then be used to specifically identify equivalent δ -endotoxins with the characteristic insecticidal activity by immunoprecipitation, enzyme linked
30 immunoassay (ELISA), or Western blotting.

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A further method for identifying the δ -endotoxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are nucleotide sequences having a detectable label. As is well known in the art, if the probe molecule and nucleic acid sample hybridize when together in a sample by forming hydrogen bonds between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or substantially similar or homologous at least along the length of the probe. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying insecticidal δ -endotoxin genes of the subject invention.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probes of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, by methods currently known to an ordinarily skilled artisan, and perhaps by other methods which may become known in the future.

The potential variations in the probes listed is due, in part, to the redundancy of the genetic code. Because of the redundancy of the genetic code, more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins. Therefore different nucleotide sequences can code for a particular amino acid. Thus, the amino acid sequences of the *B. thuringiensis* δ -endotoxins and peptides, and the plastid targeting peptides and the polynucleotides which code for them, can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein or peptide.

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA.

In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by various publications. As will be appreciated, the technique typically employs a

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phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage or plasmids containing an M13 origin of replication. These phage are readily commercially available and their use is generally well known to those skilled in the art.

5 Modification and changes may be made in the structure of the peptides of the present invention and DNA segments which encode them and still obtain a functional molecule that encodes a protein or peptide with desirable characteristics. The biologically functional equivalent peptides, polypeptides, and proteins contemplated herein should possess about 80% or greater sequence similarity, preferably about 85% or greater sequence similarity, and most preferably
10 about 90% or greater sequence similarity, to the sequence of, or corresponding moiety within, the fundamental Cry3B amino acid sequence.

 The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. In particular embodiments of the invention, mutated crystal proteins are contemplated to be useful for increasing the
15 insecticidal activity of the protein, and consequently increasing the insecticidal activity and/or expression of the recombinant transgene in a plant cell. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the codons given in readily available amino acid codon tables.

 For example, certain amino acids may be substituted for other amino acids in a protein
20 structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like
25 properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

 In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a
30 protein is generally understood in the art (Kyte and Doolittle, 1982, incorporate herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to

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the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Polynucleotides encoding δ -endotoxins derived from *B. thuringiensis* are known by those skilled in the art, to be poorly expressed when incorporated into the nuclear DNA of transgenic plants (reviewed by Diehn *et al.*, 1996). Preferably, a nucleotide sequence encoding the δ -endotoxin of interest is designed essentially as described in U. S. Patent 5,500,365 and 5,689,052. Examples of nucleotide sequences useful for expression include but are not limited to, *cry3B* (SEQID NO:5), *cry3Bb1* (SEQID NO:1), *cry3Bb2* (SEQID NO:3), *v11231* (SEQID NO:7), *11231mv1* (SEQID NO:9), and *11231mv2* (SEQID NO:11).

Peptides, polypeptides, and proteins biologically functionally equivalent to Cry3B include amino acid sequences containing conservative amino acid changes in the fundamental sequence shown in SEQID NO:2, SEQID NO:4, SEQID NO:8., SEQID NO:10, and SEQID NO:12 (Cry3Bb1, Cry3Bb2, v11231, 11231mv1, 11231mv2, Cry3Bb.11231, or Cry3Bb.11098, etc). In such amino acid sequences, one or more amino acids in the fundamental sequence is

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(are) substituted with another amino acid(s), the charge and polarity of which is similar to that of the native amino acid, *i.e.* a conservative amino acid substitution, resulting in a silent change.

Substitutes for an amino acid within the fundamental polypeptide sequence can be selected from other members of the class to which the naturally occurring amino acid belongs.

5 Amino acids can be divided into the following four groups: (1) acidic amino acids; (2) basic amino acids; (3) neutral polar amino acids; and (4) neutral non-polar amino acids.

Representative amino acids within these various groups include, but are not limited to: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cyteine, cystine, tyrosine, asparagine, and glutamine; (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

Conservative amino acid changes within the fundamental polypeptide sequence can be made by substituting one amino acid within one of these groups with another amino acid within the same group. Biologically functional equivalents of Cry3B can have 10 or fewer conservative amino acid changes, more preferably seven or fewer conservative amino acid changes, and most preferably five or fewer conservative amino acid changes. The encoding nucleotide sequence (gene, plasmid DNA, cDNA, non-naturally occurring, or synthetic DNA) will thus have corresponding base substitutions, permitting it to encode biologically functional equivalent forms of Cry3B.

20 The present invention provides methods and compositions for expressing Coleopteran inhibitory Cry3B *B. thuringiensis* δ -endotoxins or amino acid sequence variants thereof at unexpectedly high levels in transgenic plants. The disclosed methods and compositions may exploit any of the DNA constructs disclosed as well as any of the transformation vectors disclosed herein. The contemplated methods and compositions enable Cry3Bb δ -endotoxins or amino acid sequence variants thereof to be expressed in plants without negatively affecting the recovery of agronomic qualities of transgenic plants. The inventions described herein also enables expression of Cry3B δ -endotoxins and variants at levels up to 500 times higher than that achieved by previous methods and compositions.

30 The methods described here thus enables plants expressing Cry3B or variants to be used as either an alternative or supplement to plants expressing other Cry proteins such as a Cry3B variant, a Cry3A or Cry3D or variant, CryET33 and CryET34 or variants thereof, a CryET70 or variant, a

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CryET29 or variant, a Cry6A or Cry6B or variant, a Cry8B or variant, insecticidal acyl lipid hydrolases, combinations of amino acid oxidases and tedanalactam synthases, and other insecticidal proteins such as VIP1 and VIP3 and various combinations isolated from *Heterorhabdus*, *Photorhabdus*, and *Xenorhabdus* species for both control and resistance management of key insect pests, including *Ostrina* sp, *Diatraea* sp., *Diabrotica*, *Helicoverpa* sp, *Spodoptera* sp in *Zea mays*; *Heliothis virescens*, *Helicoverpa* sp, *Pectinophora* sp. in *Gossypium hirsutum*; and *Anticarsia* sp, *Pseudoplusia* sp, *Epinotia* sp in *Glycine max*. It is also contemplated that the methods described may be used to dramatically increase expression of *B. thuringiensis* δ -endotoxins including and related to Cry3, thus increasing its effectiveness against target pests and decreasing the likelihood of evolved resistance to these proteins. In one embodiment of the present invention, a Cry3 δ -endotoxin is expressed. Target pests of this protein and their common hosts are shown below in Table 1.

Table 1

Target Pests Affected by Coleopteran Active (Inhibitory) Cry3B δ -Endotoxin and Common Plant Hosts of Those Pests

Pests	Hosts
<i>Leptinotarsa decemlineata</i> (Colorado Potato Beetle)	Potato
<i>Diabrotica barberi</i> (Northern Corn Rootworm)	Corn
<i>Diabrotica undecimpunctata</i> (Southern Corn Rootworm)	Corn
<i>Diabrotica virgifera</i> (Western Corn Rootworm)	Corn
<i>Anthonomus grandis</i> (Boll Weevil)	Cotton
<i>Triboleum castaneum</i> (Red Flour Beetle)	Wheat
<i>Popilla japonica</i> (Japanese Flour Beetle)	Wheat

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Antibodies were required for studies comparing expression of various Cry3 coding sequences, so polyclonal serum was generated as follows. Cry3 Bt crystals were collected from a sporulated fermentation of *Bacillus thuringiensis* recombinant strain 11037 expressing native Cry3Bb. Crystals were solubilized in 100 mM sodium carbonate buffer, pH10.5, to give a concentration of 2.7 mg protein per mL as measured by a colorimetric bicinchoninic acid assay (Smith et al, 1985). A sample was diluted to a concentration of 0.4 mg/mL and mixed with an equal volume of Freund's complete adjuvant. A 1 milliliter inoculum of this mixture was used for the first intradermal injection into a rabbit. A first bleed

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was collected two weeks later. Subsequent injections of Cry3Bb protein designed to boost the immune titer were prepared by mixing equal volumes of 0.2 mg/mL protein with equal volumes of Freund's incomplete adjuvant. 1 milliliter injections were administered at four week intervals, and additional bleeds were obtained every two weeks. Immune serum adequate for analytical purposes was prepared from rabbit #783 after purification over a Protein A Sepharose CL-4B affinity chromatography according to the manufacturers' instructions (Sigma Chemical Co, St. Louis, Missouri) and concentrated to 1 milligram of IgG protein per milliliter and stored in the dark at 4°C. A sample of this antiserum was conjugated to alkaline phosphatase enzyme for subsequent use in quantitative ELISA assays.

Leaf and root samples were collected from plants expressing Cry3Bb variant proteins 11231, 11084, 11098, and 11247. Extracts of plant samples were prepared as follows. Plant tissue, root or leaf parts, was harvested and weighed on a gram scale. Leaf tissue was mixed with 20 parts TBA buffer, weight to volume. Root tissue was mixed with 10 parts TBA buffer, weight to volume. Tissues were ground into an emulsion using a Wheaton™ overhead grinder and stored on ice or at -20°C. 250 microliters of rabbit anti-Cry3Bb antiserum diluted 1:1000 in carbonate coating buffer, pH9.6, was distributed onto each well of a 96-well microtiter plate and incubated overnight at 4°C. The plate was then washed with PBST (3 x 5 min). Tissue extract samples were loaded in duplicate at 20 microliters per well and at varying dilutions in order to obtain a value within a standard curve established using Cry3Bb variant 11231. Plates were incubated overnight at 4°C, then washed with PBST three times, five minutes each time. 50 microliters of the rabbit anti-Cry3B alkaline phosphatase conjugated polyclonal antibody was added to each well, followed by the addition of 180 uL of PBST containing 1% PVP-40 (Sigma). After overnight incubation, plates were washed with PBST (3 X 5 min) and developed with alkaline phosphatase color development solution consisting of 20 mg para-nitrophenyl phosphate in 25 mL diethanolamine, pH9.8, 200 uL/well). Plates were read at $\lambda 405$ after 15-20 minutes, using a quadratic curve fit to a protein standard curve where the optical density of the highest standard was approximately 1.00.

5.0 EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate

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that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5.1 Example 1 - Isolation, Characterization, and Identification of Cry3 proteins and genes, and Construction of Amino Acid Sequence Variants Thereof

Means for identifying and characterizing Coleopteran toxic gene products are well documented in the art, and methods for isolating, characterizing and identifying the genes which encode such gene products are also well known in the art. In addition, the means for producing amino acid sequence variants of such Coleopteran toxic δ -endotoxin proteins are also well known. In particular, Van Rie et al. (US Patent No. 5,659,123; 1997) identify Cry3A and D toxins which exhibit Coleopteran inhibitory properties, and also set forth a method for identifying mutants which can be constructed which have reduced insecticidal activity with reference to the wild type protein. Van Rie et al. describe how those particular mutants can be further manipulated to identify amino acid sequence variant toxins which exhibit increased insecticidal activity with reference to the wild type protein. English et al. (WO 99/31248) describe other methods and compositions, in particular for Cry3B, which enable the identification of Cry3 encoding genes and gene products and the methods which can be used to construct and identify amino acid sequence variants exhibiting improved insecticidal activity with reference to that of the wild type Cry3 protein. Several coding sequences used herein were derived from those described in English et al. and the proteins produced from these coding sequences represent in particular the variants 11231 or 11098 as described therein.

5.2

Example 2. Construction of monocot plant expression vectors for the Cry3Bb variants Design of cry3Bb variant genes for plant expression

For efficient expression of the Cry3Bb variants in transgenic plants, the gene encoding the variants must have a suitable sequence composition (Diehn et al, 1996). One example of such a sequence is shown for the v11231 gene (SEQID NO:7) which encodes the 11231 variant of the Cry3Bb protein (SEQID NO: 8) exhibiting *Diabroticus* activity. This gene was derived via mutagenesis (Kunkel, 1985) of a Cry3Bb synthetic gene (SEQID NO:5) encoding a protein

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essentially homologous to the protein encoded by the native Cry3Bb gene (Gen Bank Accession Number m89794; SEQID NO:1) . The following oligonucleotides were used in the mutagenesis of the original Cry3Bb synthetic gene (SEQID NO:5) to create the v11231 gene (SEQID NO:7)

- 5 Oligo #1: TAGGCCTCCATCCATGGCAAACCCTAACAATC (SEQID NO: 40)
Oligo #2: TCCCATCTTCCTACTTACGACCCTGCAGAAATACGGTCCAAC_(SEQID NO:41)
Oligo #3: GACCTCACCTACCAAACATTCGATCTTG (SEQID NO: 42)
Oligo #4: CGAGTTCTACCGTAGGCAGCTCAAG (SEQID NO:43)

10 Construction of cry3Bb monocot plant expression vector

To place the Cry3Bb variant gene v11231 in a vector suitable for expression in monocotyledonous plants (i.e. under control of the enhanced Cauliflower Mosaic Virus 35S promoter and linker to the hsp70 intron followed by a nopaline synthase polyadenylation site as in Brown and Santino US patent number 5,424,412; 1995), the vector pMON19469 was digested
15 with NcoI and EcoRI. The larger vector band of approximately 4.6 kb was isolated after electrophoresis of the digestion products through an agarose gel, purified, and ligated with T4 DNA ligase to the NcoI-EcoRI fragment of approximately 2 kb containing the v11231 gene (SEQID NO:7). The ligation mix was transformed into a useful laboratory strain of *E. coli*, and carbenicillin resistant colonies were recovered. Plasmid DNA was recovered by miniprep DNA
20 procedures from subsequent overnight cultures of carbenicillin resistant colonies selected into broth containing antibiotics. This DNA was subjected to restriction endonuclease analysis with enzymes such as NcoI and EcoRI, NotI, and PstI to identify clones containing the v11231 coding sequence fused to the hsp70 intron under control of the enhanced CaMV35S promoter. Clones identified as such were designated as pMON33708.

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To place the v11231 gene in a vector suitable for recovery of stably transformed and insect resistant plants, the 3.75 kb NotI restriction fragment from pMON33708 containing the lysine oxidase coding sequence fused to the hsp70 intron under control of the enhanced CaMV35S promoter was isolated and purified after extraction from an agarose gel. This
30 fragment was ligated with pMON30460 treated with NotI and calf intestinal alkaline phosphatase. pMON30460 contains the neomycin phosphotransferase coding sequence under

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control of the CaMV35S promoter. Kanamycin resistant colonies were obtained by transformation of this ligation mix into *E. coli* and colonies containing the appropriate band were identified by restriction endonuclease digestion and designated as pMON33710. Restriction enzymes such as NotI, EcoRV, HindIII, NcoI, EcoRI, and BglII were used to identify the appropriate clones containing the NotI fragment of pMON33708 in the NotI site of pMON30460 (i.e. pMON33710) in the orientation such that both genes are in tandem (i.e. the 3' end of the v11231 expression cassette is linked to the 5' end of the nptII expression cassette). Expression of the v11231 protein by pMON33710 in corn protoplasts was confirmed by electroporation of pMON33710 covalently closed circular plasmid DNA into protoplasts followed by protein blot and ELISA analysis. This vector can be introduced into the genomic DNA of corn embryos by particle gun bombardment followed by paromomycin selection to obtain corn plants expressing the v11231 gene essentially as described in Brown and Santino US patent number 5,424,412. In this example, the vector was introduced into immature embryo scutella (IES) of maize via co-bombardment along with with a plasmid conferring hygromycin resistance, followed by hygromycin selection, and regeneration. Transgenic corn lines expressing the v11231 protein were identified by ELISA analysis scoring for both the presence and amount of v11231 protein present in each extract sample. Plants were selfed and allowed to go to seed. Progeny seed were cured and planted to produce seedling corn plants which were subsequently tested for protection from *Diabroticus* feeding.

In plant performance of Cry3Bb variant 11231

Transformed corn plants expressing Cry3Bb variant 11231 protein were challenged with western corn rootworm (WCR) larvae in both a seedling and 10 inch pot assay. The transformed genotype was A634, where the progeny of the R0 cross by A634 was evaluated. Observations included effect on larval development (weight), root damage rating (RDR), and protein expression. The transformation vector containing the Cry3Bb variant gene was pMON33710. Treatments included the positive and negative iso-populations for each event and an A634 check.

The seedling assay consisted of the following steps; i. single seeds were placed in 1 oz cups containing potting soil; ii. at spiking, each seedling was infested with 4 neonate larvae, and iii. after infestation, seedlings were incubated for 7 days at 25°C, 50% RH, and 14:10 (L:D) photo

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period. Adequate moisture was added to the potting soil during the incubation period to maintain seedling vigor.

The 10 inch pot assay consisted of the following steps; i. single seeds were placed in 10 inch pots containing potting soil; ii. at 14 days post planting, each pot was infested with 800 eggs which have been pre-incubated such that hatch would occur 5-7 days post infestation; and iii. after infestation, plants were incubated for 4 weeks under the same environmental conditions as the seedling assay. Pots were both sub & top irrigated daily.

For the seedling assay, on day 7 plants were given a root damage rating (Table 1.) and surviving larvae were weighed. Also at this time, Cry3Bb protein concentrations in the roots were determined by ELISA.

Table 1. Root Damage Rating Scale for seedling assay.

RDR	0 = no visible feeding
	1 = very light feeding
	2 = light feeding
	3 = moderate feeding
	4 = heavy feeding
	5 = very heavy feeding

Results of the seedling assay are shown in Table 2. Plants expressing Cry3Bb protein were completely protected by WCR feeding, where surviving larvae within this treatment had not grown. Mean larval weights ranged from 2.03 - 2.73 mg for the non-expressing treatments, where the surviving larval average weight was 0.11 mg on the expressing Cry3Bb treatment. Root damage ratings were 3.86 and 0.33 for the non-expressing and expressing iso-populations, respectively. Larval survival ranged from 75 - 85 % for the negative and check treatments, where only 25 % of the larvae survived on the Cry3Bb treatment.

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Table 2. Effect of Cry3Bb expressing plants on WCR larvae in a seedling assay.

Event	Treatment	N	Plants		N	Larvae	
			Root (ppm)	RDR \pm SD		% Surv	Mean \pm SD Wt.(mg)
16	Negative	7	0.0	3.86 \pm 0.65	21	75	2.73 \pm 1.67
16	Positive	3	29.01	0.33 \pm 0.45	3	25	0.11 \pm 0.07
A634	Check	4	0.0	--	13	81	2.03 \pm 0.83

For the 10 inch pot assay, at 4 weeks post infestation plant height was recorded and a root damage rating was given (Iowa 1-6 scale; Hills, T.M. and D.C. Peters. 1971; A method of evaluating post planting insecticide treatments for control of western corn rootworm larvae. Journal of Economic Entomology 64: 764-765.).

Results of the 10 inch pot assay are shown in Table 3. Plants expressing Cry3Bb protein had significantly less feeding damage and were taller than the non-expressing plants. Event 16, the higher of the two expressing events provided nearly complete control. The negative treatments had very high root damage ratings indicating very high insect pressure. The positive mean root damage ratings were 3.4 and 2.2 for event 6 & 16, respectively. Mean RDR for the negative treatment was 5.0 & 5.6.

Table 3. Effect of Cry3Bb expressed in corn in controlling WCR larval feeding in a 10 inch pot assay.

Event	Treatment	N	Root		Plant Height (cm)
			(ppm)	RDR \pm SD	
6	Negative	7	0.0	5.0 \pm 1.41	49.7 \pm 18.72
6	Positive	5	7.0	3.4 \pm 1.14	73.9 \pm 8.67
16	Negative	5	0.0	5.6 \pm 0.89	61.2 \pm 7.75
16	Positive	5	55.0	2.2 \pm 0.84	83.8 \pm 27.15

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In summary, corn plants expressing Cry3Bb protein have a significant biological effect on WCR larval development as seen in the seedling assay. When challenged with very high infestation levels, plants expressing the Cry3Bb protein were protected from WCR larval feeding damage as illustrated in the 10 inch pot assay.

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Example 3 - Increased Expression of a Cry3Bb protein in transgenic maize

Expression of a Cry3Bb protein was compared in corn plants transformed with standard or preferred Cry3Bb expression vectors. Plants transformed with the improved vectors

10 consistently demonstrated significantly higher levels of expression of Cry3Bb when compared to plants transformed with the standard Cry3Bb vectors. A standard Cry3Bb plant expression vector pMON33710 contains an expression cassette composed of an enhanced CaMV35S promoter sequence (P-CaMV.35S, SEQID NO:29), a *Zea mays* Hsp70 intron sequence (I-Zm.Hsp70, SEQID NO:33), a non-naturally occurring sequence encoding Cry3Bb variant

15 protein v11231 (Bt.cry3Bb.v11231, SEQID NO: 7), and a nopaline synthase transcription termination and polyadenylation sequence (T-AGRtu.nos, SEQID NO:34). Another standard Cry3Bb plant expression vector pMON33709 contains an expression cassette composed of an enhanced CaMV35S promoter sequence (P-CaMV.35S, SEQID NO:29), a *Zea mays* Hsp70 intron sequence (I-Zm.Hsp70, SEQID NO:33), a *Zea mays* CTP encoding sequence (TS-

20 Zm.rbc1, SEQID NO:25), a non-naturally occurring sequence encoding Cry3Bb variant protein v11231 (Bt.cry3Bb.v11231, SEQID NO:7), and a nopaline synthase transcription termination and polyadenylation sequence (T-AGRtu.nos, SEQID NO:34). The plant expression vector pMON25097 is improved compared to pMON33710 as judged by Cry3Bb expression levels *in planta*, and contains an expression cassette comprising a non-naturally occurring CaMV35S

25 AS4 promoter sequence (P-CaMV.AS4, SEQID NO:30), a wheat chlorophyll A/B binding protein untranslated leader sequence (L-Ta.hcb1, SEQID NO:31), a rice actin intron sequence (I-Os.Act1, SEQID NO:32), and a non-naturally occurring sequence encoding Cry3Bb variant protein 11231mv1 (11098) (Bt.cry3Bb.11231mv1, SEQID NO:9) linked to a wheat heat shock Hsp17 transcription termination and polyadenylation sequence (T-Ta.Hsp17, SEQID NO:35).

30 Another preferred vector is pMON25096, which contains an expression cassette (SEQID NO:17) comprising a non-naturally occurring CaMV35S AS4 promoter sequence (P-

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CaMV.AS4, SEQID NO:30), a wheat chlorophyll A/B binding protein untranslated leader sequence (L-Ta.hcb1, SEQID NO:31), a rice actin intron sequence (I-Os.Act1, SEQID NO:32), a *Zea mays* CTP encoding sequence (TS-Zm.rbc1, SEQID NO:25), and a non-naturally occurring sequence encoding Cry3Bb variant protein 11231mv1 (Bt.cry3Bb.11231mv1, SEQID NO:9) linked to a wheat heat shock Hsp17 transcription termination and polyadenylation sequence (T-Ta.Hsp17, SEQID NO:35). All vectors contain an identical cassette linked to the Cry3Bb expression cassette which confers paromomycin resistance to transformed plant tissue. This resistance cassette consists of an enhanced CaMV35S promoter sequence, and a neomycin phosphotransferase coding sequence linked to a nopaline synthase transcription termination and polyadenylation sequence. A summary of the standard and improved vectors is presented in Table 4. Transgenic corn plants resistant to paromomycin were derived essentially as described in U. S. Patent 5,424,412 (1995).

Table 4
Plant Expression Vector Summary

Vector	Expression Cassette	Selection Cassette
pMON33709	35S/HSP70/ZmRBC/v11231/NOS	e35S/nptII /nos
pMON33710	e35S/HSP70/11231v/nos	e35S/nptII /nos
pMON33722	AS4/TaCAB/OsAct1/ZmRBC/v11231/tahsp17	e35S/nptII /nos
pMON33723	AS4//TaCAB/OsAct1/v11231/tahsp17	e35S/nptII /nos
pMON25096	AS4/TaCAB/OsAct1/ZmRBC/11231mv1/tahsp17	e35S/nptII /nos
pMON25097	AS4/TaCAB/OsAct1/11231mv1/tahsp17	e35S/nptII /nos
pMON33741	AS4/TaCAB/OsAct1/11231mv2/tahsp17	e35S/nptII /nos
pMON33748	e35S/TaCAB/OsAct1/11231mv2/tahsp17	e35S/nptII /nos

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Maize leaf protoplasts were electroporated with standard vectors (pMON33709 or pMON33710) or improved vectors (pMON33722, pMON33723, pMON25096, pMON25097, pMON33741) as described (Sheen, Plant Cell 2:1027-1038, 1990) and transient expression of Cry3Bb variant proteins was compared by ELISA and Western Blot analysis methods. The ELISA used a rabbit anti-Cry3B chromatography purified IgG capture antibody raised against Cry3B 11231, a sample of that antibody conjugated to alkaline phosphatase as the secondary detecting antibody, and a purified Cry3Bb native protein as a standard. Comparison of the ratio of Cry3Bb to neomycin phosphotransferase (Npt II) expression levels by ELISA indicated that approximately two-fold increases in the normalized expression levels of Cry3Bb variant protein 11231 were obtained with improved vectors pMON33723 and pMON33722 relative to the standard vectors pMON33710 and pMON33709, respectively. (Expt. 1, Table 5). Differences in Cry3Bb expression are directly ascribed to the improved expression cassette in the improved vectors rather than to differences in protoplast electroporation efficiency since expression of Cry3Bb protein is normalized to Npt II produced by the identical linked *nptII* gene present in all vectors. The most preferred improved vectors such as pMON25096, pMON25097, and pMON33741 expressed approximately 10-fold higher normalized levels of Cry3Bb and variant Cry3Bb protein than the preferred improved vectors such as pMON33722 or pMON33723 (Table 5, Expt. 2, 3). Finally, the equally preferred pMON33741 and pMON25097 vectors yielded roughly equivalent normalized Cry3Bb expression (Table 5, Expt. 4)

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Table 5

Transient Cry3Bb and Cry3BbVariant Expression in Corn Leaf Protoplasts (normalized to NptII expression)		
Expt. 1	pMON33710 5.79	pMON33723 12.3
	pMON33709 2.7	pMON33722 7.7
Expt. 2	pMON33722 1.9	pMON25096 26.2
	pMON33723 3.7	pMON25097 37.5
Expt.3	pMON33723 30	pMON33741 319
Expt. 4	pMON33741 20	pMON25097 25

Since the improved expression cassette in pMON25097 encodes the Cry3Bb 11231mv1 (11098) variant toxin, and the standard cassette in pMON33710 encodes the Cry3Bb v11231 variant which differ by a single amino acid, the intrinsic immunoreactivity of the two proteins in the ELISA assay was compared. Subsequent ELISA experiments with Cry3Bb v11231 and 11231mv1 (11098) variant proteins produced in and purified from *B. thuringiensis* indicate that the two proteins have similar levels of immunoreactivity. Consequently, the observed increase in levels of Cry3Bb 11231mv1 (11098) protein produced from the expression cassette in pMON25097 is due to increased expression levels rather than a difference in immunoreactivity. Protein blot analyses confirm that the increased level of cross reactive material produced in maize protoplasts from the improved Cry3Bb expression cassette in pMON25097 were due to increased accumulation of an approximately 60,000 Mr protein immunoreactive with Cry3B antiserum that also co-migrates with Cry3Bb variant 11231 protein produced in a recombinant *cry-* *B. thuringiensis* strain from pEG7174. Equally preferred and improved Cry3Bb variant protein expression cassettes in pMON33741 and pMON33748 that encode Cry3Bb.11231 also

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exhibit increased expression levels of Cry3Bb relative to expression observed from the standard cassette in pMON33710. These results confirm that expression differences are due to the improved compositions disclosed herein rather than to differences in the intrinsic immunoreactivity of the different variants.

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Root tissue from transgenic plants in the R₀ stage independently obtained after transformation with an improved vectors (pMON33723, 25097, ,) or with a standard vector (pMON 33710) was subjected to quantitative analysis of Cry3Bb protein levels by a quantitative ELISA assay. Comparison of Cry3Bb or Cry3Bb protein variant expression levels in improved and standard vector transformed corn plants show that Cry3Bb.11231 variant expression does not exceed 50 ppm in the standard pMON33710 transgenics while Cry3Bb.11098 (11231mv1) expression in the improved pMON25097 transgenics is frequently higher than 50 ppm (Table 6). Protein blot analyses confirm that the increased level of cross reactive material produced by pMON25097 (improved) were due to increased accumulation of an approximately Mr 60,000 protein that migrates with Cry3Bb1 standard from *B. thuringiensis*. Other improved Cry3Bb protein variant expression cassettes found in pMON33741 and 33748 also consistently yield select independently transformed events (ITE's) with Cry3Bb protein variant levels greater than 100 PPM whereas the standard vectors have never given rise to ITE's with greater than 50 PPM of Cry3Bb protein variant (Table 7). High level expression is evident in both the H99 and A634 maize genotypes, indicating that the compositions disclosed herein have broad utility to many varieties of commercially cultivated maize. Such select high expressing Cry3 protein variant lines obtained with the vectors described herein are expected to be especially advantageous in conferring high levels of protection to insect feeding damage and in reducing the incidence of insect resistance to Cry3 insecticidal proteins.

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Table 6
Comparison of Cry3Bb Expression in R₀ Corn Transformed with Standard and Improved Cry3Bb Protein
Variant Expression Cassettes

Cry3B Expression Level (ppm)						
Vector (genotype)	Total Events	5-10 ppm	10-50 ppm	50-100 ppm	100-200 ppm	>200 ppm
L25097						
A634	45	3	7			3
H99	589	32	36	5	3	5
L33710						
A634	22	2	2			
H99	336	13	15			
L33723						
A634	0					
H99	67	6	9			

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Table 7
Cry3Bb Expression in R₀ Corn Transformed with Improved Cry3Bb Protein
Variant Expression Cassettes

Cry3B Expression Level (ppm)						
Vector	Total Events	5-10 ppm	10-50 ppm	50-100 ppm	100-200 ppm	>200 ppm
L25097						
A634	112	7	4	5	1	4
H99	45	1	4	2		
L33741						
H99	108	11	5	2		4
L33748						
A634	82	1	11	2	2	1
H99	209	23	13	3	3	11

Progeny derived from corn plants transformed with both the standard (pMON33709 and pMON33710) and preferred (pMON25096, 25097, 33722, 33723, 33726, 33741, and 33748)

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cassettes expressing 10 ppm or more of Cry3Bb protein were further tested for resistance to Corn Rootworm (CRW) feeding damage in greenhouse or growth chamber based bioassays as previously described (English et al., WO 99/31248). Corn Rootworm resistant transgenic corn plants were obtained from essentially all of the preferred vectors (Table 8). For example, the improved pMON25096 vector was used to generate 89 independently transformed events (ITE's), 14 independent pMON25096 F₁ progeny lines expressing 10 ppm or more of Cry3Bb and 7 F₁ progeny lines displaying significant levels of CRW resistance (an RDR rating \geq 3.5 on a rating scale of 0-6). In contrast, not a single event with a RDR rating \leq 3.5 was obtained from 12 of the standard pMON33710 cassette F₁ progeny lines expressing 10 PPM or more of Cry3Bb protein variant. Failure to obtain CRW resistant lines with either of the standard vectors (pMON33709 or pMON33710) was not due to insufficient numbers of ITE's as over 300 ITE's from each of these two vectors were generated and screened for CRW resistant F₁ progeny. Far fewer ITE's were generated with preferred vectors such as pMON33722, pMON33723, and pMON25096, yet all ultimately gave rise to CRW resistant F₁ progeny lines.

Table 8

Numbers of CRW resistant independent transformation events obtained with the standard and improved Cry3Bb Protein Variant expression cassettes

Expressi on cassette	Genotyp e	Total Number of ITE's	Number of ITE's Tested	Number and Percent of ITEs with RDR \leq 3.5
L33709	H99	318	11	0
L33710	H99	336	10	0
	A634	22	2	0
L25096	H99	52	4	2 (50%)
	A634	37	10	5 (50%)

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L25097	H99	634	17	10
				(59%)
	A634	157	18	8 (44%)
L33722	H99	107	10	6 (60%)
L33723	H99	93	7	3 (43%)
L33726	H99	65	6	5 (83%)
	A634	10	0	
L33727	H99	86	0	
	A634	1	1	0
33736A BI	H99	3	3	2 (67%)
L33741	H99	108	1	0
L33748	H99	223	6	3 (50%)
	A634	82	7	4 (57%)
L33749 ABI	H99	73	14	13 (93%)

In examples provided herein, experimental evidence that substantially equivalent compositions based on the improvements disclosed herein yield equivalent improvements in performance relative to the previously disclosed standards. More specifically, we demonstrate that improved compositions encoding both the Cry3Bb. 11098 and Cry3Bb. 11231 variants both yield equivalently improved performance relative to the previously disclosed standard

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compositions encoding Cry3Bb.11231. It thus follows that use of other Cry3B variants with specific biological activities that are greater than or equal to Cry3Bb.11098 or Cry3Bb.11231 is contemplated by and within the scope of this invention. For example, improved vector compositions encoding Cry3Bb variants include 11231, 11084, 11098, 11247, and others as set forth in English et al., US Application Serial No.'s 08/993,170, 08/993,722, 08/993,755, and 08/996,441, all filed Dec. 18, 1997 can be derived from pMON25095 using standard mutagenesis procedures in a manner essentially equivalent to the construction of pMON33740.

5.4

Example 4- Preferred Expression Cassettes Confer Resistance to CRW damage in Field Tests

5 Corn plants genetically modified to express Cry3Bb protein variants derived from the preferred vectors pMON33722, pMON33723, pMON25096, and pMON25097 were evaluated in the field for control of western corn rootworm, *Diabrotica vergifera vergifera* LeConte (WCR). None of the corn plants transformed with the standard vectors were advanced to field testing as none displayed adequate Corn Rootworm control in greenhouse tests (Example 3. Table 8). The efficacy trials were held at a Monsanto research farm in Jerseyville, Illinois and at the Northern Grain Insects Research Laboratory, USDA ARS research station in Brookings, South Dakota. These trials serve to evaluate performance of the preferred cassettes in the field under heavy insect pressure and to compare their performance to the current commercially available insecticides .

15 Seventeen independent transformation events (ITE) were selected for field evaluation based on greenhouse performance. The amount of seed available for the field evaluation varied for each ITE. Of these 17 events, only seven were planted at the Brookings research station. The field design for the Brookings' location was a randomized complete block (RCB) with 2 replications, where each plot was a single row containing a maximum of 30 plants. All 17 ITE's were planted at the Jerseyville location, where the design was a RCB with a maximum of 4 replications, 1 row plots each, where the number of replications depended on the seed available from each ITE. Because of this, the number of replications at Jerseyville ranged from two to four. Additional

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treatments included an untreated check (nontransgenic corn) and commercial insecticides, including Counter®, Lorsban®, and Force®. The insecticide treatments were only at the Jerseyville location. The insecticides were applied as an eight inch band at planting using the recommended rates.

5 Planting dates were May 28th and June 3rd for the Jerseyville & Brookings, respectively. The study was performed as follows; plots were infested with CRW eggs at planting with 1,600 eggs per foot of row, approximately 800 eggs per plant. At the V1 - V2 plant growth stage, plants were analyzed for presence of the Cry3Bb protein variant expression using an ELISA. Plants
10 negative for the gene were culled from the plot.

At the end of the CRW larval feeding stage, when maximum damage would have occurred, all remaining plants in each plot were evaluated for root feeding injury using a 1 - 6 root damage rating (RDR) scale described by Hills and Peters (1971). The RDR scale is as follows;

15

Root Damage Rating:

1. No feeding scars
2. Visible feeding scars, but no roots pruned to within 4 cm of the stalk
3. One or more nodal roots pruned to within 4 cm of the stalk, but less than one nodes worth of
20 roots
4. One node worth of pruned roots
5. Two nodes worth of pruned roots
6. Three or more nodes worth of pruned roots

25 On July 25th and August 3rd the field trials were evaluated at Jerseyville and Brookings, respectively. The average RDR's for all treatments are illustrated in Table 9. Of the seventeen ITE's evaluated, 16 ITE's controlled CRW feeding, ≤ 3.0 RDR. Two of the three chemical standards had a RDR less than 3.0. Force® had a root damage rating of 3.2. Except for one ITE, WCR20, all treatments were significantly better than the checks ($p < .01$) but did not differ
30 significantly from each other. Figure one illustrates the difference in larval feeding damage between a transgenic CRW resistant plant and an untreated check.

Even though the ITE's did not differ significantly from the chemical standards with respect to root damage rating, the amount of feeding injury observed on roots from the insecticide
35 treatments were greater than the roots expressing Monsanto's proprietary gene. The lack of

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difference between root damage rating is an artifact of the root rating scale, where this scale is based on “pruned” roots. Hills and Peters describe a pruned root as being less than 4 cm in length due to CRW feeding. Therefore, root masses without a “pruned” root but visible feeding scars are given a rating of 2. Roots outside of the zone of protection from the insecticide treatments had many more feeding scars and in most cases the root tips were destroyed as compared to the ITE’s. Unlike the insecticide treatments, the transgenic plants express the CRW resistant gene throughout the entire root mass. But because the mechanism for control of the transgenic plant is orally mediated, a minimum amount of feeding is required to control any further injury by the CRW larvae. This minimal feeding requirement resulted in a RDR of 2.

In summary, corn plants expressing Cry3Bb protein variants were fully protected from CRW larval feeding. This level of protection eliminates the need for an insecticide treatment. Insecticides, including organophosphates, carbamates and pyrethroids are incorporated into the soil on over 16 million corn acres annually to control CRW. CRW resistance technology has the potential to significantly reduce the current exposure level of these insecticides to the environment. The benefits of shifting away from soil insecticides to a transgenic approach are impressive and include a reduction in potential human health and safety risks, reduced direct impacts on nontarget organisms, reduced contamination of surface and ground water supplies, decreased pesticide container disposal problems, and general compatibility with other pest management and agronomic programs.

Table 9.

Corn rootworm root feeding damage (RDR) means for corn independent transformation events containing Monsanto’s proprietary CRW resistant gene.

Root Damage Rating (RDR)			
Treatment	Jerseyville	Brookings	Average (RDR)
pMON 25097-1	2.3	1.9	2.1
pMON 33722-1	2.6	2.3	2.5
pMON 33723-1	2.6	2.9	2.8
pMON 33723-2	2.6	2.0	2.3
pMON33722-2	2.5	1.9	2.2
pMON 25096-1	2.8	2.5	2.7
pMON 25097-2	2.5	2.3	2.4

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pMON 25096-2	2.4	n/a	2.4
pMON 25097-3	2.6	n/a	2.6
pMON 25096-3	2.2	n/a	2.2
pMON 25097-4	2.2	n/a	2.2
pMON 25096-4	2.6	n/a	2.6
pMON 33723-3	2.5	n/a	2.5
pMON 25097-5	3.0	n/a	3.0
pMON 25097-6	4.0	n/a	4.0
pMON 25097-7	2.2	n/a	2.2
pMON 33722-3	2.6	n/a	2.6
COUNTER ®	2.4	n/a	2.4
LORSBAN ®	2.4	n/a	2.4
FORCE ®	3.2	n/a	3.2
CHECK	4.1	4.1	4.1

5.5

Example 5 - Transformation of Tobacco Chloroplast with a *cry3B* gene

Recombinant plants can be produced in which only the mitochondrial or chloroplast DNA has been altered to incorporate the molecules envisioned in this application. Promoters which function in chloroplasts have been known in the art (Hanley-Bowden et al., Trends in Biochemical Sciences **12**:67-70, 1987). Methods and compositions for obtaining cells containing chloroplasts into which heterologous DNA has been inserted have been described, for example by Daniell et al. (U.S. Pat. No. 5,693,507; 1997) and Maliga et al. (U.S. Pat. No. 5,451,513; 1995). A vector can be constructed which contains an expression cassette from which a Cry3B protein could be produced. A cassette could contain a chloroplast operable promoter sequence driving expression of a *cry3B* crystal protein gene, constructed in much the same manner as other polynucleotides herein, using thermal amplification methodologies, restriction endonuclease digestion, and ligation etc. A chloroplast expressible gene would provide a promoter and a 5' untranslated region from a heterologous gene or chloroplast gene such as *psbA*, which would provide for transcription and translation of a DNA sequence encoding a Cry3B protein in the chloroplast; a DNA sequence encoding Cry3B protein; and a transcriptional and translational termination region such as a 3' inverted repeat region of a chloroplast gene that could stabilize an expressed *cry3B* mRNA. Expression from within the chloroplast would enhance *cry3B* gene product accumulation. A host cell containing chloroplasts or plastids can be transformed with the expression cassette and then the resulting

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cell containing the transformed chloroplasts can be grown to express the Cry3B protein. A cassette may also include an antibiotic, herbicide tolerance, or other selectable marker gene in addition to the *cry3B* gene. The expression cassette may be flanked by DNA sequences obtained from a chloroplast DNA which would facilitate stable integration of the expression cassette into the chloroplast genome, particularly by homologous recombination. Alternatively, the expression cassette may not integrate, but by including an origin of replication obtained from a chloroplast DNA, would be capable of providing for replication of the heterologous *cry3B* gene in the chloroplast. Plants can be generated from cells containing transformed chloroplasts and can then be grown to produce seeds, from which additional plants can be generated. Such transformation methods are advantageous over nuclear genome transformation, in particular where chloroplast transformation is effected by integration into the chloroplast genome, because chloroplast genes in general are maternally inherited. This provides environmentally "safer" transgenic plants, virtually eliminating the possibility of escapes into the environment. Furthermore, chloroplasts can be transformed multiple times to produce functional chloroplast genomes which express multiple desired recombinant proteins, whereas nuclear genomic transformation has been shown to be rather limited when multiple genes are desired. Segregational events are thus avoided using chloroplast or plastid transformation. Unlike plant nuclear genome expression, expression in chloroplasts or plastids can be initiated from only one promoter and continue through a polycistronic region to produce multiple peptides from a single mRNA.

The expression cassette would be produced in much the same way that other plant transformation vectors are constructed. Plant chloroplast operable DNA sequences can be inserted into a bacterial plasmid and linked to DNA sequences expressing desired gene products, such as Cry3B proteins, so that Cry3B protein is produced within the chloroplast, obviating the requirement for nuclear gene regulation, capping, splicing, or polyadenylation of nuclear regulated genes, or chloroplast or plastid targeting sequences. An expression cassette comprising a *cry3B* gene, which is either synthetically constructed or a native gene derived directly from a *B. thuringiensis* genome or a *B. thuringiensis* episomal element, would be inserted into a restriction site in a vector constructed for the purpose of chloroplast or plastid transformation. The cassette would be flanked upstream by a chloroplast or plastid functional promoter and downstream by a chloroplast or plastid functional transcription and translation termination

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sequence. The resulting cassette would be incorporated into the chloroplast or plastid genome using well known homologous recombination methods.

Alternatively, chloroplast or plastid transformation could be obtained by using an autonomously replicating plasmid or other vector capable of propagation within the chloroplast or plastid. One means of effectuating this method would be to utilize a portion of the chloroplast or plastid genome required for chloroplast or plastid replication initiation as a means for maintaining the plasmid or vector in the transformed chloroplast or plastid. A sequence enabling stable replication of a chloroplast or plastid epigenetic element would easily be identified from random cloning of a chloroplast or plastid genome into a standard bacterial vector which also contains a chloroplast or plastid selectable marker gene, followed by transformation of chloroplasts or plastids and selection for transformed cells on an appropriate selection medium. Introduction of an expression cassette as described herein into a chloroplast or plastid replicable epigenetic element would thus provide an effective means for localizing a Cry3B *B. thuringiensis* δ -endotoxin to the chloroplast or plastid.

5.6

Example 6: Targeting Cry3Bb or Variant Cry3Bb Protein to Plastids

Improved expression by targeting recombinant insecticidal protein to the chloroplast may result in tissues which are light exposed and which accumulate mature chloroplasts as a result. Improving expression in leaf tissue to inhibit leaf-feeding pests susceptible to the insecticidal protein could be advantageous. To test this, two plasmids, pMON33709 and pMON33710 were constructed which were isogenic with respect to all elements with the exception of a plastid or chloroplast targeting sequence linked in frame to the insecticidal Cry3Bb improved variant in pMON33709. R_0 corn plants were recovered and were shown to contain and express the transgene by ELISA. Six pMON33709 lines and sixteen pMON33710 lines were recovered which expressed the transgene in both the root and the leaves. Leaf and root tissue were recovered and analyzed for the presence and amount of Cry3Bb variant protein, measured in parts per million. The results are shown in Table 10.

Table 10.

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Comparison of Non-Targeted and Plastid Targeted Leaf vs Root Expression of Cry3Bb Variant v11231 in R₀ Corn Transformation Events

R0 #	Event #	Construct	Tissue	ppm 11231(ug/g tissue)
R053608	2027-05-01	L33709	Leaf	14.69
R053608	2027-05-01	L33709	Root	3.97
R053621	2028-06-06	L33709	Leaf	22.65
R053621	2028-06-06	L33709	Root	0.10
R053643	2029-03-09	L33709	Leaf	1.05
R053643	2029-03-09	L33709	Root	3.83
R053675	2028-03-06	L33709	Leaf	7.13
R053675	2028-03-06	L33709	Root	2.23
R053688	2028-04-02	L33709	Leaf	56.80
R053688	2028-04-02	L33709	Root	9.83
R053690	2028-04-02	L33709	Leaf	98.69
R053690	2028-04-02	L33709	Root	6.38
R053708	2027-01-02	L33710	Leaf	12.79
R053708	2027-01-02	L33710	Root	4.94
R053781	2028-02-19	L33710	Leaf	8.47
R053781	2028-02-19	L33710	Root	4.72
R053785	2027-04-06	L33710	Leaf	21.97
R053785	2027-04-06	L33710	Root	7.20
R053799	2028-01-16	L33710	Leaf	12.41
R053799	2028-01-16	L33710	Root	6.19
R053800	2028-01-16	L33710	Leaf	5.69
R053800	2028-01-16	L33710	Root	3.32
R053801	2028-01-16	L33710	Leaf	16.19
R053801	2028-01-16	L33710	Root	7.80
R053824	2027-01-11	L33710	Leaf	6.93
R053824	2027-01-11	L33710	Root	10.35
R053838	2030-08-12	L33710	Leaf	14.32
R053838	2030-08-12	L33710	Root	5.64
R053857	2030-08-08	L33710	Leaf	12.70
R053857	2030-08-08	L33710	Root	3.97
R053858	2028-02-32	L33710	Leaf	2.33
R053858	2028-02-32	L33710	Root	4.15
R053859	2028-02-32	L33710	Leaf	9.39
R053859	2028-02-32	L33710	Root	5.76
R053904	2027-02-03	L33709	Leaf	226.05
R053904	2027-02-03	L33709	Root	1.55
R053923	2029-01-08	L33710	Leaf	12.16
R053923	2029-01-08	L33710	Root	11.77
R053924	2029-01-08	L33710	Leaf	10.74
R053924	2029-01-08	L33710	Root	7.94
R053928	2029-01-05	L33710	Leaf	14.86
R053928	2029-01-05	L33710	Root	3.84
R053929	2029-01-05	L33710	Leaf	15.04
R053929	2029-01-05	L33710	Root	3.49

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All but one pMON33709 line (Ro53643) produced between 3 to 15 times more insecticidal protein in the leaves than in the root tissue. The one line that produced less in the leaves also produced less than 1 ppm in the root, whereas the other lines produced up to almost 100 ppm in the leaves. The amount of Cry3Bb variant protein expressed was even more variable
5 in the non-targeted lines derived from pMON33710 transformation events which were determined to be expressing the recombinant protein in both leaf and root tissues. While most of these lines produced more protein in the leaves than in the roots, some also produced more in the roots, but the difference between the amount produced in the roots in those improved root-expressors was less substantial than in the single pMON33709 targeted event. Also, the range of
10 expression levels was less pronounced in the non-targeted events with one exception. Surprisingly, one line (Ro53904) produced substantially more protein in the leaves than was observed in any other line, targeted or non-targeted. This line would be expected to be a candidate for a commercial line directed to protection against Coleopteran pests which feed on leaf tissues. Conversely, lines such as Ro53923 would be expected to be optimum candidates for
15 protecting corn plants against root-feeding pests such as corn rootworms.

The data in summary indicates that targeting the Bt Cry3B protein to the plastid or chloroplast improves the accumulation of the protein in leaf tissue but not in root tissue, and improves the overall expression of the protein in leaves in plants transformed with such constructs as compared to the levels of expression observed in root tissues in those same plants.

20 In view of the above, it will be seen that the several advantages of the invention are achieved and other advantageous results attained. As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.
25 In addition, all references referred to in this application are herein incorporated by reference in their entirety.

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Claims:

1. A plant comprising a polynucleotide sequence comprising an expression cassette containing a linear arrangement of genetic sequences which function together in plant cells to achieve improved expression of at least an insecticidal portion of a protein or amino acid sequence variant thereof from a nucleic acid coding sequence in said plant cells, said protein or
5 variant being derived from a *Bacillus thuringiensis* Cry3 δ -endotoxin, said variant exhibiting an insecticidal activity toxic to a Coleopteran insect pest which is at least equivalent to said Cry3, wherein said genetic sequences comprise a promoter operably linked in linear sequence to an untranslated leader, an intron, said nucleic acid coding sequence, and a transcription termination
10 and polyadenylation sequence.
2. The plant according to claim 1 wherein said endotoxin protein or amino acid sequence variant thereof is a Cry3B protein or variant thereof, said variant exhibiting an insecticidal activity at least equivalent to said Cry3B.
15
3. The plant according to claim 2 wherein said endotoxin protein or amino acid sequence variant thereof is a Cry3Bb protein or variant thereof, said variant exhibiting an insecticidal activity at least equivalent to said Cry3Bb.
- 20 4. The plant according to claim 3 wherein said endotoxin protein or amino acid sequence variant thereof is a Cry3Bb2 protein or variant thereof, said variant exhibiting an insecticidal activity at least equivalent to said Cry3Bb2.
5. The plant according to claim 1 wherein said endotoxin protein or amino acid
25 sequence variant thereof is a protein as set forth in SEQID NO:2 or SEQID NO:4 or a variant thereof, said variant exhibiting an insecticidal activity at least equivalent to the protein as set forth in SEQ ID NO:2.
6. The plant according to claim 5 wherein said endotoxin protein or amino acid
30 sequence variant thereof is a protein or a variant thereof selected from the group consisting of SEQID NO:4, SEQID NO:8, SEQID NO:10, and SEQID NO:12, said variant exhibiting an insecticidal activity at least equivalent to the protein as set forth in SEQID NO:4.

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7. The plant according to claim 1, wherein said genetic sequence comprising a promoter is selected from the group consisting of CaMV-35S-AS4 promoter, CaMV-e35S promoter, and CaMV-35S promoter, and POX promoter.

5 8. The plant according to claim 1, wherein said genetic sequence comprising a promoter is selected from the group consisting of SEQID NO:30 and SEQID NO:29.

9. The plant according to claim 1 wherein said genetic sequence comprising an untranslated leader is the wheat chlorophyll ab binding protein untranslated leader.

10

10. The plant according to claim 1 wherein said genetic sequence comprising an untranslated leader is SEQID NO:31.

11. The plant according to claim 1 wherein said genetic sequence comprising an
15 intron is selected from the group consisting of rice actin intron and HSP70 intron.

12. The plant according to claim 1 wherein said genetic sequence comprising an intron is selected from the group consisting of SEQID NO:32 and SEQID NO:33.

20 13. The plant according to claim 1 wherein said genetic sequence comprising a transcription termination and polyadenylation sequence is selected from the group consisting of Agrobacterium tumefaciens nopaline synthase transcription termination and polyadenylation sequence and the wheat hsp17 transcription termination and polyadenylation sequence element.

25 14. The plant according to claim 1 wherein said genetic sequence comprising a transcription termination and polyadenylation sequence is selected from the group consisting of SEQID NO:34 and SEQID NO:35.

15. The plant according to claim 1 wherein said genetic sequence comprising said
30 nucleic acid coding sequence is selected from the group consisting of SEQID NO:1, SEQID NO:3, SEQID NO:7, SEQID NO:9, and SEQID NO:11.

16. The plant according to claim 1 wherein said expression cassette is selected from the group consisting of SEQID NO:15, SEQID NO:38, SEQID NO:19, SEQID NO:21, and
35 SEQID NO:23.

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17. The plant according to claim 1 selected from the group consisting of a monocotyledonous plant and a dicotyledonous plant.

5 18. The plant according to claim 17 which is a monocotyledonous plant.

19. The plant according to claim 1 or a progeny of said plant, wherein said plant or said progeny comprise said polynucleotide sequence.

10 20. A seed from the plant or progeny of claim 19.

21. A plant germinated from the seed of claim 20.

22. A plant comprising a polynucleotide sequence comprising an expression cassette
15 containing a linear arrangement of genetic sequences which function together in plant cells to achieve improved expression of a *Bacillus thuringiensis* Cry3 insecticidal δ -endotoxin protein or amino acid sequence variant thereof toxic to a Coleopteran insect pest from a nucleic acid coding sequence in said plant cells, said variant exhibiting an insecticidal activity at least equivalent to said Cry3, wherein said genetic sequences comprise a promoter operably linked in
20 linear sequence to an untranslated leader, an intron, a nucleotide sequence encoding a plastid or chloroplast targeting peptide linked in frame and adjacent to said nucleic acid coding sequence, and a transcription termination and polyadenylation sequence.

23. The plant of claim 17 wherein said genetic sequence comprising a nucleotide
25 sequence encoding a plastid or chloroplast targeting peptide is a *Zea mays* ribulose bis phosphate carboxylase synthase small subunit chloroplast targeting sequence.

24. The plant of claim 17 wherein said genetic sequence comprising a nucleotide
sequence encoding a plastid or chloroplast targeting peptide is SEQID NO:25.

30

25. A plant comprising a polynucleotide sequence comprising an expression cassette containing a linear arrangement of genetic sequences which function together in plant cells to achieve expression from a nucleic acid sequence encoding an insecticidal *Bacillus thuringiensis* Cry3 δ -endotoxin protein or amino acid sequence variant thereof toxic to a Coleopteran insect
35 pest feeding on said plant, said variant exhibiting an insecticidal activity at least equivalent to

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said Cry3, said genetic sequences comprising a promoter operably linked in linear sequence to an untranslated leader, an intron, said nucleic acid coding sequence, and a transcription termination and polyadenylation sequence, wherein said expression of said protein or variant in plant cells of said plant is improved to levels of protein or variant sufficient to protect said plant from Coleopteran pest infestation and sufficient to delay the onset of insect resistance to said protein or variant.

26. The plant of claim 20 wherein said improved expression provides levels of protein or variant in plant cells from about 200 to about 500 parts per million of total plant protein.

27. The plant of claim 20, wherein said improved expression provides levels of protein or variant in plant cells from about 100 to about 200 parts per million of total plant protein.

28. The plant of claim 20, wherein said improved expression provides levels of protein or variant in plant cells from about 50 to about 100 parts per million of total plant protein.

29. The plant of claim 20, wherein said improved expression provides levels of protein or variant in plant cells from about 10 to about 50 parts per million of total plant protein.

30. The plant of claim 20, wherein said improved expression provides levels of protein or variant in plant cells from about 5 to about 10 parts per million of total plant protein.

31. The plant of claim 20, wherein the percentage of plants obtained exhibiting said improved expression providing levels of protein or variant in said plant cells which are greater than about 200 parts per million of total cell protein is from about 0.5 to about 5 percent of all plants obtained after transformation and selection of events using said expression cassette to produce transgenic events.

32. The plant of claim 20, wherein the percentage of plants obtained exhibiting said improved expression providing levels of protein or variant in said plant cells which are greater than about 100 parts per million of total cell protein is from about 0.5 to about 6 percent of all

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plants obtained after transformation and selection using said expression cassette to produce transgenic events.

33. The plant of claim 20, wherein the percentage of plants obtained exhibiting said improved expression providing levels of protein or variant in said plant cells which are greater than about 50 parts per million of total cell protein is from about 0.5 to about 8 percent of all plants obtained after transformation and selection using said expression cassette to produce transgenic events.

34. The plant of claim 20, wherein the percentage of plants obtained exhibiting said improved expression providing levels of protein or variant in said plant cells which are greater than about 10 parts per million of total cell protein is from about 0.5 to about 18 percent of all plants obtained after transformation and selection using said expression cassette to produce transgenic events.

35. A method for producing a transgenic plant comprising the steps of:

a) introducing into the DNA of a plant cell or plant tissue a polynucleotide sequence comprising an expression cassette containing a linear arrangement of genetic sequences which function together in plant cells to achieve expression from a nucleic acid sequence encoding an insecticidal *Bacillus thuringiensis* Cry3 δ -endotoxin protein or amino acid sequence variant thereof toxic to a Coleopteran insect pest feeding on said plant, said variant exhibiting an insecticidal activity at least equivalent to said Cry3, said genetic sequences comprising a promoter operably linked in linear sequence to an untranslated leader, an intron, said nucleic acid coding sequence, and a transcription termination and polyadenylation sequence;

b) growing said plant cell or plant tissue in selective media to obtain a transgenic plant containing said polynucleotide sequence heritably incorporated into said DNA; and

c) selecting a plant expressing said protein or variant;

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wherein said selected plant exhibits improved expression of said protein or variant in said transgenic plant, said improved expression being improved to levels of protein or variant sufficient to protect said plant from Coleopteran pest infestation and sufficient to delay the onset of insect resistance to said protein or variant when produced in said transgenic plant.

36. The method according to claim 35 wherein said step introducing said polynucleotide into the DNA of said plant cell or tissue is accomplished by nucleic acid coated ballistic particle bombardment, electroporation, or transformation competent *Agrobacterium tumefaciens* transformed with a plasmid containing said polynucleotide sequence.

37. The method according to claim 36 wherein said transgenic plant is a monocot or a dicot.

38. The method of claim 37 wherein said transgenic plant is a monocot.

39. The method of claim 38 wherein said monocot is selected from the group consisting of corn, wheat, barley, rice, oats, grasses, and bananas.

40. The method according to claim 35 wherein said selecting step is accomplished using a polynucleotide sequence comprising an expression cassette containing a promoter which functions in plants operably linked to nucleotide sequence encoding a selectable marker protein linked to a plant operable 3' transcription termination and polyadenylation sequence.

41. The method according to claim 40 wherein the selectable marker protein is selected from the group consisting of herbicide tolerance proteins, antibiotic resistance proteins, proteins which catalyze substrates to generate visually observed colored products, and proteins which catalyze substrates to generate fluorescent or luminescent products.

The method according to claim 40 wherein the selectable marker protein is selected from the group consisting of NptII, GUS, LUX, Hyg, esterase, PhnO, EPSPS, and GOX.

42. A vector comprising a polynucleotide sequence as set forth in claim 1 for use in transforming a plant, plant cell, or plant tissue, wherein said vector is selected from the group

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consisting of a plasmid, a bacmid, an artificial chromosome, a linear single or double stranded DNA or RNA fragment, and a virus genome.

43. An isolated and purified polynucleotide sequence comprising an expression
5 cassette containing a linear arrangement of genetic sequences which function together in plant
cells to achieve improved expression of at least an insecticidal portion of a protein or amino acid
sequence variant thereof from a nucleic acid coding sequence in said plant cells, said protein or
variant being derived from a *Bacillus thuringiensis* Cry3 δ -endotoxin, said variant exhibiting an
insecticidal activity toxic to a Coleopteran insect pest which is at least equivalent to said Cry3,
10 wherein said genetic sequences comprise a promoter operably linked in linear sequence to an
untranslated leader, an intron, said nucleic acid coding sequence, and a transcription termination
and polyadenylation sequence, and wherein said expression cassette improves the expression of
the protein or variant in a transgenic plant and, improves the number of transgenic events
observed to express the protein or variant above a threshold level when said polynucleotide
15 sequence is used for generating transformed plants.

44. An isolated and purified polynucleotide sequence comprising one or more
expression cassettes, each cassette comprising genetic sequence elements which function in
plant cells to express a desired *Bacillus thuringiensis* δ -endotoxin insecticidal protein, chimera,
20 fusion, or variant thereof from a nucleic acid coding sequence, wherein said coding sequence is
linked upstream to a promoter sequence element, an untranslated leader sequence element, an
intron sequence element, and said coding sequence is linked downstream to a transcription
termination and polyadenylation sequence element, wherein said expression cassette improves
the expression of the protein or variant in a transgenic plant and improves the percentage of
25 transgenic events observed to express the desired protein above a threshold level when using
said polynucleotide sequence for generating transformed plants.

45. A method for controlling Coleopteran insect infestation in a field of crop plants
comprising providing a transgenic plant on which said Coleopteran insect feeds, said transgenic
30 plant comprising a polynucleotide sequence comprising an expression cassette containing a
linear arrangement of genetic sequences which function together in plant cells to achieve
improved expression of at least an insecticidal portion of a protein or amino acid sequence
variant thereof from a nucleic acid coding sequence in said plant cells, said protein or variant
being derived from a *Bacillus thuringiensis* Cry3 δ -endotoxin, said variant exhibiting an
35 insecticidal activity toxic to a Coleopteran insect pest which is at least equivalent to said Cry3,

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wherein said genetic sequences comprise a promoter operably linked in linear sequence to an untranslated leader, an intron, said nucleic acid coding sequence, and a transcription termination and polyadenylation sequence.

- 5 46. The method according to claim 45 wherein the genetic sequences which function together in said transgenic plant improves the expression of the protein or variant in a transgenic plant and improves the percentage of transgenic events observed to express the desired protein above a threshold level when using said polynucleotide sequence for generating transformed plants.

10

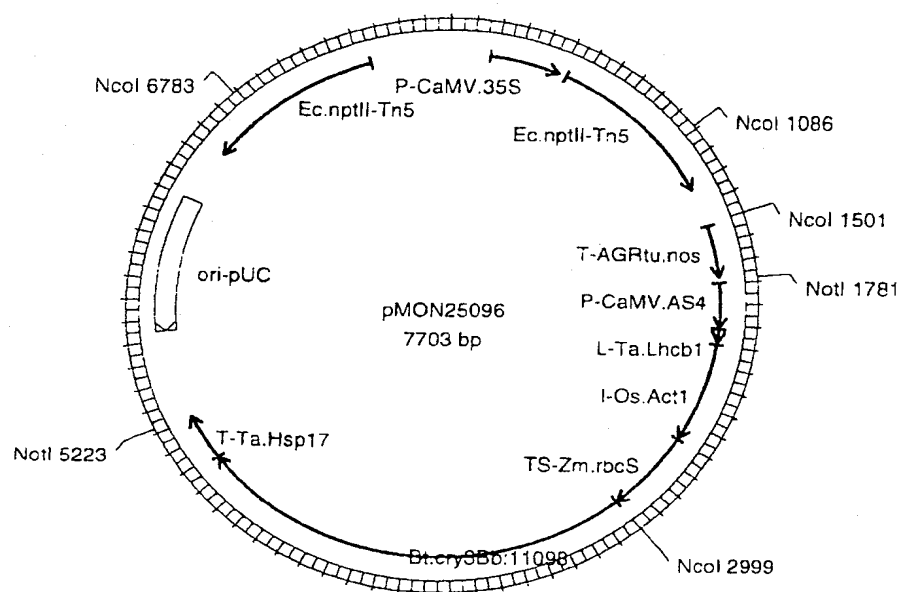
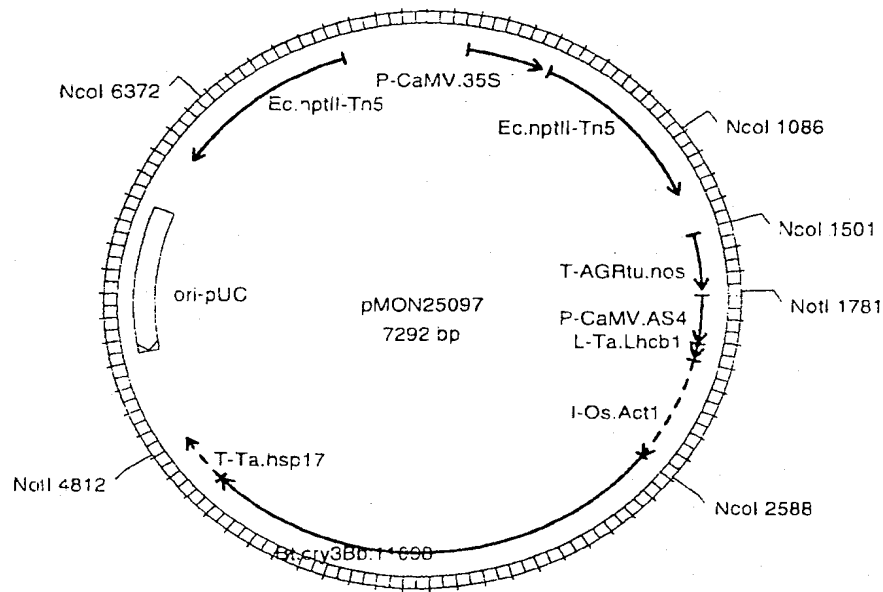


Figure 1

**Figure 2**

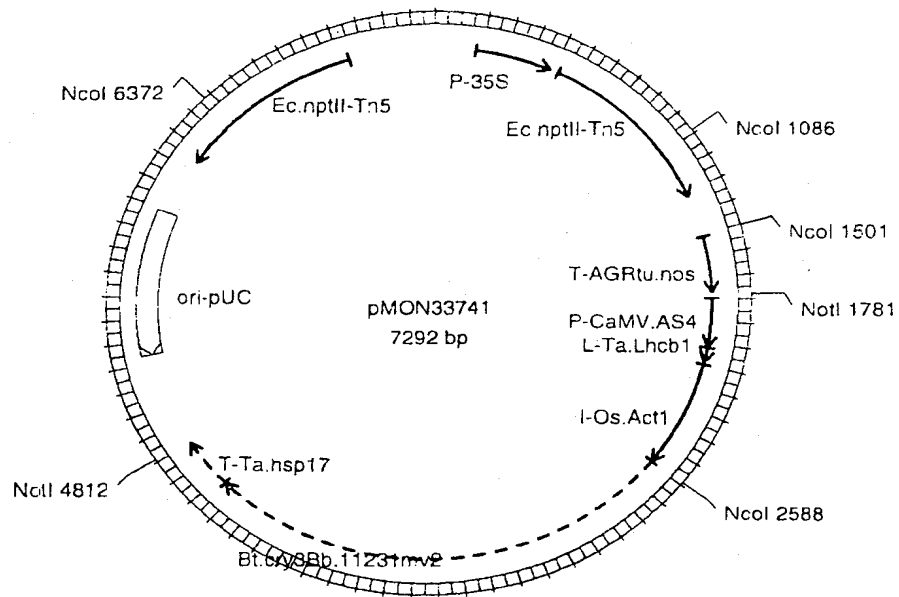


Figure 3

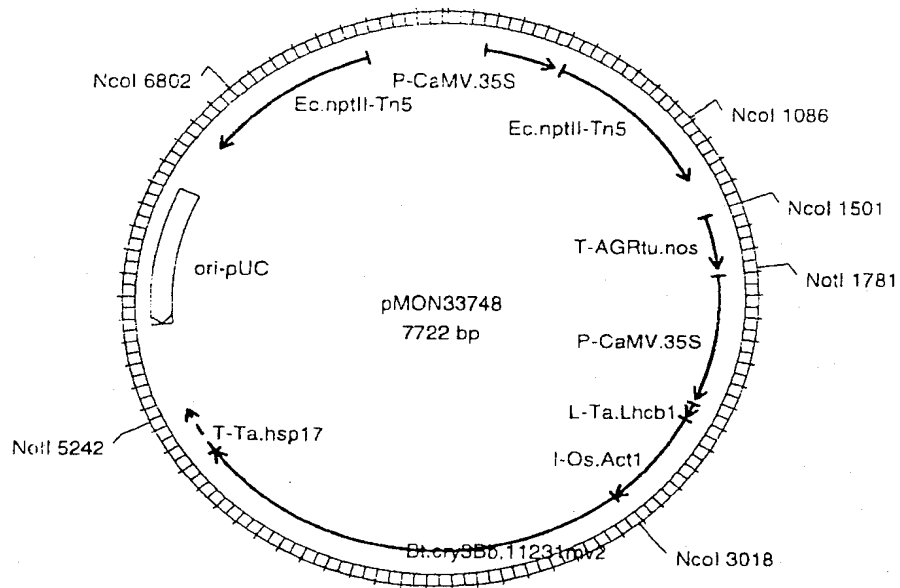


Figure 4

Figure 5A

```

atg gcc aac ccc aac aat cgc tcc gag cac gac acg atc aag gtc 47
Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val
1 5 10 15

acc ccc aac tcc gag ctc cag acc aac cac aac cag tac ccg ctg gcc 95
Thr Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala
20 25 30

gac aac ccc aac tcc acc ctg gaa gag ctg aac tac aag gag ttc ctg 143
Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu
35 40 45

cgc atg acc gag gac tcc tcc acg gag gtc ctg gac aac tcc acc gtc 191
Arg Met Thr Glu Asp Ser Ser Thr Thr Glu Val Leu Asp Asn Ser Thr Val
50 55 60

aag gac gcc gtc ggc acc ggc atc tcc gtc gtt ggg cag atc ctg ggc 239
Lys Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly
65 70 75

gtc gtt ggc gtc ccc ttc gca ggt gct ctc acc tcc ttc tac cag tcc 287
Val Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser
80 85 90 95

ttc ctg aac acc atc tgg ccc tcc gac gcc gac ccc tgg aag gcc ttc 335
Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe
100 105 110

atg gcc caa gtc gaa gtc ctg atc gac aag aag atc gag gag tac gcc 383
Met Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala
115 120 125

aag tcc aag gcc ctg gcc gag ctg caa ggc ctg caa aac aac ttc gag 431
Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu
130 135 140

gac tac gtc aac gcg ctg aac tcc tgg aag aag acg cct ctg tcc ctg 479
Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu
145 150 155

cgc tcc aag cgc tcc cag ggc cgc atc cgc gag ctg ttc tcc cag gcc 527

```

Figure 5B

Arg Ser Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala	175
160	165
gag tcc cac ttc cgc aac tcc atg ccg tcc ttc gcc gtc tcc aag ttc	575
Glu Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe	190
180	185
gag gtc ctg ttc ctg ccc acc tac gcc cag gct gcc aac acc cac ctc	623
Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu	205
195	200
ctg ttg ctg aag gac gcc cag gtc ttc gcc gag gaa tgg ggc tac tcc	671
Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser	220
210	215
tcg gag gac gtc gcc gag ttc tac cgt cgc cag ctg aag ctg acc caa	719
Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln	235
225	230
cag tac acc gac cac tgc gtc aac tgg tac aac gtc gcc ctg aac ggc	767
Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly	255
240	245
ctg agg ggc tcc acc tac gac gca tgg gtc aag ttc aac cgc ttc cgc	815
Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg	270
260	265
agg gag atg acc ctg acc gtc ctg gac ctg atc gtc ctg ttc ccc ttc	863
Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe	285
275	280
tac gac atc cgc ctg tac tcc aag ggc gtc aag acc gag ctg acc cgc	911
Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg	300
290	295
gac atc ttc acg gac ccc atc ttc ctg ctg acc ctg cag aag tac	959
Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr	315
305	310
ggt ccc acc ttc ctg tcc atc gag aac tcc atc cgc aag ccc cac ctg	1007
Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu	335
320	325

Figure 5C

```

ttc gac tac ctc cag ggc atc gag ttc cac acg cgc ctg agg cca ggc 1055
Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly
340 345 350

tac ttc ggc aag gac tcc ttc aac tac tgg tcc ggc aac tac gtc gag 1103
Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu
355 360 365

acc agg ccc tcc atc ggc tcc tcg aag acg atc acc tcc cct ttc tac 1151
Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr
370 375 380

ggc gac aag tcc acc gag ccc gtc cag aag ctg tcc ttc gac ggc cag 1199
Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln
385 390 395

aag gtc tac cgc acc atc gcc aac acc gac gtc gcg gct tgg ccg aac 1247
Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn
400 405 410 415

ggc aag gtc tac ctg ggc gtc acg aag gtc gac ttc tcc cag tac gat 1295
Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp
420 425 430

gac cag aag aat gaa acc tcc acc cag acc tac gac tcc aag cgc aac 1343
Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn
435 440 445

aat ggc cac gtc tcc gcc cag gac tcc atc gac cag ctg ccg cct gag 1391
Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu
450 455 460

acc act gac gag ccc ctg gag aag gcc tac tcc cac cag ctg aac tac 1439
Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr
465 470 475

gcg gag tgc ttc ctg atg caa gac cgc agg ggc acc atc ccc ttc ttc 1487
Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe
480 485 490 495

acc tgg acc cac cgc tcc gtc gac ttc ttc aac acc atc gac gcc gag 1535

```

Figure 5D

```

Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu
500                                     505
1583
aag atc acc cag ctg ccc gtg gtc aag gcc tac gcc ctg tcc tcg ggt
Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly
515                                     520
1631
gcc tcc atc att gag ggt cca gcc ttc acc ggt gcc aac ctg ctg ttc
Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe
530                                     535
1679
ctg aag gag tcc tcg aac tcc atc gcc aag ttc aag gtc acc ctg aac
Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn
545                                     550
1727
tcc gct gcc ttg ctg caa cgc tac cgc gtc cgc atc cgc tac gcc tcc
Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser
560                                     565
1775
acc acg aac ctg cgc ctg ttc gtc cag aac tcc aac aat gac ttc ctg
Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu
580                                     585
1823
gtc atc tac atc aac aag acc atg aac aag gac gat gac ctg acc tac
Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr
595                                     600
1871
cag acc ttc gac ctc gcc acc acg aac tcc aac atg gcc ttc tcg ggc
Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly
610                                     615
1919
gac aag aat gaa ctg atc att ggt gct gag tcc ttc gtc tcc aat gaa
Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu
625                                     630
1961
aag atc tac atc gac aag atc gag ttc atc ccc gtc cag ctg
Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
640                                     645

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Figure 6A

```

atg gcc aac ccc aac aat cgc tcc gag cac gac acg atc aag gtc      47
Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val
1      5      10      15
acc ccc aac tcc gag ctc cag acc aac cac aac cag tac ccg ctg gcc      95
Thr Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala
20      25      30
gac aac ccc aac tcc acc ctg gaa gag ctg aac tac aag gag ttc ctg      143
Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu
35      40      45
cgc atg acc gag gac tcc tcc acg gag gtc ctg gac aac tcc acc gtc      191
Arg Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val
50      55      60
aag gac gcc gtc ggc acc ggc atc tcc gtc gtt ggc cag atc ctg ggc      239
Lys Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly
65      70      75
gtc gtt ggc gtc ccc ttc gca ggt gct ctc acc tcc ttc tac cag tcc      287
Val Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser
80      85      90      95
ttc ctg aac acc atc tgg ccc tcc gac gcc gac ccc tgg aag gcc ttc      335
Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe
100      105      110
atg gcc caa gtc gaa gtc ctg atc gac aag aag atc gag gag tac gcc      383
Met Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala
115      120      125
aag tcc aag gcc ctg gcc gag ctg caa ggc ctg caa aac aac ttc gag      431
Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu
130      135      140
gac tac gtc aac gcg ctg aac tcc tgg aag aag acg cct ctg tcc ctg      479
Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu
145      150      155

```

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Figure 6B

cgc tcc aag cgc tcc cag gac cgc atc cgc gag ctg ttc tcc cag gcc 527
 Arg Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala 175
 160 165 170
 gag tcc cac ttc cgc aac tcc atg ccg tcc ttc gcc gtc tcc aag ttc 575
 Glu Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe 190
 180 185
 gag gtc ctg ttc ctg ccc acc tac gcc cag gct gcc aac acc cac ctg 623
 Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu 205
 195 200
 ctg ttg ctg aag gag gcc cag gtc ttc gcc gag gaa tgg gcc tac tcc 671
 Leu Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser 220
 210 215
 tcg gag gac gtc gcc gag ttc tac cgt cgc cag ctg aag ctg acc caa 719
 Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln 235
 225 230
 cag tac acc gac cac tgc gtc aac tgg tac aac gtc gcc ctg aac gcc 767
 Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly 255
 240 245
 ctg agg gcc tcc acc tac gac gca tgg gtc aag ttc aac cgc ttc cgc 815
 Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg 270
 260 265
 agg gag atg acc ctg acc gtc ctg gac ctg atc gtc ctg ttc ccc ttc 863
 Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe 285
 275 280
 tac gac atc cgc ctg tac tcc aag gcc gtc aag acc gag ctg acc cgc 911
 Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg 300
 290 295
 gac atc ttc acg gac ccc atc ttc ctg ctc acg acc ctc cag aag tac 959
 Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr 315
 305 310
 ggt ccc acc ttc ctg tcc atc gag aac tcc atc cgc aag ccc cac ctg 1007

Figure 6C

Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu 335
 320 325 330
 ttc gac tac ctc cag ggc atc gag ttc cac acg cgc ctg agg cca ggc 1055
 Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly 350
 340 345
 tac ttc ggc aag gac tcc ttc aac tac tgg tcc ggc aac tac gtc gag 1103
 Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu 365
 355 360
 acc agg ccc tcc atc ggc tcc tcg aag acg atc acc tcc cct ttc tac 1151
 Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr 380
 370 375 380
 ggc gac aag tcc acc gag ccc gtc cag aag ctg tcc ttc gac ggc cag 1199
 Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln 395
 385 390
 aag gtc tac cgc acc atc gcc aac acc gac gtc gtc ggc gct tgg ccg aac 1247
 Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn 410 415
 400 405
 ggc aag gtc tac ctg ggc gtc acg aag gtc gac ttc tcc cag tac gat 1295
 Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp 430
 420 425
 gac cag aag aat gaa acc tcc acc cag acc tac gac tcc aag cgc aac 1343
 Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn 440 445
 435
 aat ggc cac gtc tcc gcc cag gac tcc atc gac cag ctg ccg cct gag 1391
 Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu 460
 450 455
 acc act gac gag ccc ctg gag aag gcc tac tcc cac cag ctg aac tac 1439
 Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr 475
 465 470
 gcg gag tgc ttc ctg atg caa gac cgc agg ggc acc atc ccc ttc ttc 1487
 Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe

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Figure 6D

480	485	490	495
acc tgg acc cac cgc tcc gtc gac ttc ttc aac acc atc gac gcc gag			1535
Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu	500	505	510
aag atc acc cag ctg ccc gtg gtc aag gcc tac gcc ctg tcc tcg ggt			1583
Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly	515	520	525
gcc tcc atc att gag ggt cca cgc ttc acc ggt gcc aac ctg ctg ttc			1631
Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe	530	535	540
ctg aag gag tcc tcg aac tcc atc gcc aag ttc aag gtc acc ctg aac			1679
Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn	545	550	555
tcc gct gcc ttg ctg caa cgc tac cgc gtc cgc atc cgc tac gcc tcc			1727
Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser	560	565	570
acc acg aac ctg cgc ctg ttc gtc cag aac tcc aac aat gac ttc ctg			1775
Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu	580	585	590
gtc atc tac atc aac aag acc atg aac aag gac gat gac ctg acc tac			1823
Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr	595	600	605
cag acc ttc gac ctg gcc acc acg aac tcc aac atg gcc ttc tcg gcc			1871
Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly	610	615	620
gac aag aat gaa ctg atc att ggt gct gag tcc ttc gtc tcc aat gaa			1919
Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu	625	630	635
aag atc tac atc gac aag atc gag ttc atc ccc gtc cag ctg			1961
Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	640	645	650

SEQUENCE LISTING

<110> Romano, Charles P.

<120> Improved Expression of Cry3Bb in Corn

<130> 38-21(15304) Cry3Bb Improved Exp. Corn

<140> unknown

<141> 1999-08-19

<150> 60/097,150

<151> 1998-08-19

<160> 43

<170> PatentIn Ver. 2.0

<210> 1

<211> 1959

<212> DNA

<213> Bacillus thuringiensis

<220>

<221> CDS

<222> (1)..(1956)

<220>

<223> Description of Artificial Sequence: naturally
 occurring nucleotide sequence encoding a Cry3Bb1
 amino acid sequence

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Met Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
aac agt gaa ttg caa act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tca aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	

aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
ggt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt aca caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtt aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggg tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
att cgg tta tac tca aaa ggg gtt aaa aca gaa cta aca aga gac att	912
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctt aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggg att gaa ttt cat acg cgt ctt caa cct ggt tac ttt	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	

aaa tct act gaa cct gta caa aag cta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggt aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
gta tat tta ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	
aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc	1344
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly	
435 440 445	
cat gta agt gca cag gat tct att gac caa tta ccg cca gaa aca aca	1392
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr	
450 455 460	
gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa	1440
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu	
465 470 475 480	
tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg	1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp	
485 490 495	
aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att	1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile	
500 505 510	
act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc	1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser	
515 520 525	
att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa	1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys	
530 535 540	
gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca	1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala	
545 550 555 560	
gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act	1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr	
565 570 575	
aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt ctt gtc atc	1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile	
580 585 590	
tac att aat aaa act atg aat aaa gat gat gat tta aca tat caa aca	1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr	
595 600 605	
ttt gat ctg gca act act aat tct aat atg ggg ttc tcg ggt gat aag	1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys	
610 615 620	
aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc	1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile	
625 630 635 640	

tat ata gat aag ata gaa ttt atc cca gta caa ttg taa
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

1959

<210> 2
 <211> 652
 <212> PRT
 <213> Bacillus thuringiensis

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 Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35 40 45
 Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp
 50 55 60
 Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
 65 70 75 80
 Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
 85 90 95
 Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
 100 105 110
 Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
 115 120 125
 Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
 130 135 140
 Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
 145 150 155 160
 Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
 165 170 175
 His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
 180 185 190
 Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
 195 200 205
 Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
 210 215 220
 Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
 225 230 235 240
 Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
 245 250 255
 Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
 260 265 270
 Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp

275	280	285
Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300		
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320		
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335		
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350		
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365		
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380		
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400		
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415		
Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430		
Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445		
His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460		
Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 465 470 475 480		
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp 485 490 495		
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile 500 505 510		
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser 515 520 525		
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys 530 535 540		
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala 545 550 555 560		
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr 565 570 575		
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile 580 585 590		
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr 595 600 605		
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys 610 615 620		

Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
625 630 635 640

Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
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<210> 3

<211> 1959

<212> DNA

<213> *Bacillus thuringiensis*

<220>

<221> CDS

<222> (1) .. (1956)

<223> naturally occurring nucleotide sequence encoding a
Cry3Bb2 amino acid sequence

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aac agt gaa ttg cca act aac cat aat caa tat cct tta gct gac aat	96
Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cca aat tcg aca cta gaa gaa tta aat tat aaa gaa ttt tta aga atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
act gaa gac agt tct acg gaa gtg cta gac aac tct aca gta aaa gat	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gca gtt ggg aca gga att tct gtt gta ggg cag att tta ggt gtt gta	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
gga gtt cca ttt gct ggg gca ctc act tca ttt tat caa tca ttt ctt	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
gac act ata tgg cca agt gat gct gac cca tgg aag gct ttt atg gca	336
Asp Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtt gaa gta ctg ata gat aag aaa ata gag gag tat gct aaa agt	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gct ctt gca gag tta cag ggt ctt caa aat aat ttc gaa gat tat	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtt aat gcg tta aat tcc tgg aag aaa aca cct tta agt ttg cga agt	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aaa aga agc caa gat cga ata agg gaa ctt ttt tct caa gca gaa agt	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	

cat ttt cgt aat tcc atg ccg tca ttt gca gtt tcc aaa ttc gaa gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctg ttt cta cca aca tat gca caa gct gca aat aca cat tta ttg cta	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
tta aaa gat gct caa gtt ttt gga gaa gaa tgg gga tat tct tca gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	
210 215 220	
gat gtt gct gaa ttt tat cat aga caa tta aaa ctt acg caa caa tac	720
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	
225 230 235 240	
act gac cat tgt gtc aat tgg tat aat gtt gga tta aat ggt tta aga	768
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	
245 250 255	
ggt tca act tat gat gca tgg gtc aaa ttt aac cgt ttt cgc aga gaa	816
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	
260 265 270	
atg act tta act gta tta gat cta att gta ctt ttc cca ttt tat gat	864
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	
275 280 285	
gtt cgg tta tac tca aaa ggt gtt aaa aca gaa cta aca aga gac att	912
Val Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	
290 295 300	
ttt acg gat cca att ttt tca ctc aat act ctt cag gag tat gga cca	960
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	
305 310 315 320	
act ttt ttg agt ata gaa aac tct att cga aaa cct cat tta ttt gat	1008
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	
325 330 335	
tat tta cag ggt att gaa ttt cat acg cgt ctt caa cct ggt tac tct	1056
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Ser	
340 345 350	
ggg aaa gat tct ttc aat tat tgg tct ggt aat tat gta gaa act aga	1104
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg	
355 360 365	
cct agt ata gga tct agt aag aca att act tcc cca ttt tat gga gat	1152
Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp	
370 375 380	
aaa tct act gaa cct gta caa aag tta agc ttt gat gga caa aaa gtt	1200
Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val	
385 390 395 400	
tat cga act ata gct aat aca gac gta gcg gct tgg ccg aat ggc aag	1248
Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys	
405 410 415	
ata tat ttt ggt gtt acg aaa gtt gat ttt agt caa tat gat gat caa	1296
Ile Tyr Phe Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln	
420 425 430	

aaa aat gaa act agt aca caa aca tat gat tca aaa aga aac aat ggc 1344
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445

 cat gta ggt gca cag gat tct att gac caa tta cca cca gaa aca aca 1392
 His Val Gly Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460

 gat gaa cca ctt gaa aaa gca tat agt cat cag ctt aat tac gcg gaa 1440
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480

 tgt ttc tta atg cag gac cgt cgt gga aca att cca ttt ttt act tgg 1488
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495

 aca cat aga agt gta gac ttt ttt aat aca att gat gct gaa aag att 1536
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510

 act caa ctt cca gta gtg aaa gca tat gcc ttg tct tca ggt gct tcc 1584
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525

 att att gaa ggt cca gga ttc aca gga gga aat tta cta ttc cta aaa 1632
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540

 gaa tct agt aat tca att gct aaa ttt aaa gtt aca tta aat tca gca 1680
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560

 gcc ttg tta caa cga tat cgt gta aga ata cgc tat gct tct acc act 1728
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575

 aac tta cga ctt ttt gtg caa aat tca aac aat gat ttt att gtc atc 1776
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Ile Val Ile
 580 585 590

 tac att aat aaa act atg aat ata gat gat gat tta aca tat caa aca 1824
 Tyr Ile Asn Lys Thr Met Asn Ile Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605

 ttt gat ctc gca act act aat tct aat atg ggg ttc tcg ggt gat acg 1872
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Thr
 610 615 620

 aat gaa ctt ata ata gga gca gaa tct ttc gtt tct aat gaa aaa atc 1920
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640

 tat ata gat aag ata gaa ttt atc cca gta caa ttg taa 1959
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 4

<211> 652

<212> PRT

<213> Bacillus thuringiensis

<400> 4

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Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	35	40	45
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	50	55	60
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	65	70	75
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	85	90	95
Asp Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	100	105	110
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	115	120	125
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	130	135	140
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	145	150	155
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	165	170	175
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	180	185	190
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	195	200	205
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	210	215	220
Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr	225	230	235
Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg	245	250	255
Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu	260	265	270
Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp	275	280	285
Val Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile	290	295	300
Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro	305	310	315
Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp	325	330	335
Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Ser	340	345	350

Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355 360 365
 Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370 375 380
 Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385 390 395 400
 Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Ile Tyr Phe Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Gly Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Ile Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Ile Asp Asp Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Thr
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 5

<211> 1962

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic or
non-naturally occurring nucleotide sequence
encoding a Cry3Bb amino acid sequence

<220>

<221> CDS

<222> (1)..(1956)

<400> 5

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aac tct gag ttg caa act aat cac aac cag tac cca ttg gct gac aat	96
Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
cct aac agt act ctt gag gaa ctt aac tac aag gag ttt ctc cgg atg	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
acc gaa gat agc tcc act gag gtt ctc gat aac tct aca gtg aag gac	192
Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp	
50 55 60	
gct gtt gga act ggc att agc gtt gtg gga cag att ctt gga gtg gtt	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
ggg gtt cca ttc gct gga gct ttg acc agc ttc tac cag tcc ttt ctc	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
aac acc atc tgg cct tca gat gct gat ccc tgg aag gct ttc atg gcc	336
Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
caa gtg gaa gtc ttg atc gat aag aag atc gaa gag tat gcc aag tct	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
aaa gcc ttg gct gag ttg caa ggt ttg cag aac aac ttc gag gat tac	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	
gtc aac gca ctc aac agc tgg aag aaa act ccc ttg agt ctc agg tct	480
Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser	
145 150 155 160	
aag cgt tcc cag gac cgt att cgt gaa ctt ttc agc caa gcc gaa tcc	528
Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser	
165 170 175	
cac ttc aga aac tcc atg cct agc ttt gcc gtt tct aag ttc gag gtg	576
His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val	
180 185 190	
ctc ttc ttg cca aca tac gca caa gct gcc aac act cat ctc ttg ctt	624
Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu	
195 200 205	
ctc aaa gac gct cag gtg ttt ggt gag gaa tgg ggt tac tcc agt gaa	672
Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu	

210	215	220	
gat gtt gcc gag ttc tac cat agg cag ctc aag ttg act caa cag tac Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr 225 230 235 240			720
aca gac cac tgc gtc aac tgg tac aac gtt ggg ctc aat ggt ctt aga Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg 245 250 255			768
gga tct acc tac gac gca tgg gtg aag ttc aac agg ttt cgt aga gag Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu 260 265 270			816
atg acc ttg act gtg ctc gat ctt atc gtt ctc ttt cca ttc tac gac Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp 275 280 285			864
att cgt ctt tac tcc aaa ggc gtt aag aca gag ctg acc aga gac atc Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile 290 295 300			912
ttc acc gat ccc atc ttc tca ctt aac acc ctg cag gaa tac ggt cca Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro 305 310 315 320			960
act ttt ctc tcc att gag aac agc atc agg aag cct cac ctc ttc gac Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp 325 330 335			1008
tat ctg caa ggc att gag ttt cac acc agg ttg caa cct ggt tac ttc Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe 340 345 350			1056
ggt aag gat tcc ttc aac tac tgg agc gga aac tac gtt gaa acc aga Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg 355 360 365			1104
cca tcc atc gga tct agc aag acc atc act tct cca ttc tac ggt gac Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp 370 375 380			1152
aag agc act gag cca gtg cag aag ttg agc ttc gat ggg cag aag gtg Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val 385 390 395 400			1200
tat aga acc atc gcc aat acc gat gtt gca gct tgg cct aat ggc aag Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys 405 410 415			1248
gtc tac ctt gga gtt act aaa gtg gac ttc tcc caa tac gac gat cag Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln 420 425 430			1296
aag aac gag aca tct act caa acc tac gat agt aag agg aac aat ggc Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly 435 440 445			1344
cat gtt tcc gca caa gac tcc att gac caa ctt cca cct gaa acc act His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr 450 455 460			1392
gat gaa cca ttg gag aag gct tac agt cac caa ctt aac tac gcc gaa Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu 1440			

465	470	475	480	
tgc ttt ctc atg caa gac agg cgt ggc acc att ccg ttc ttt aca tgg				1488
Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp				
	485	490	495	
act cac agg tct gtc gac ttc ttt aac act atc gac gct gag aag att				1536
Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile				
	500	505	510	
acc caa ctt ccc gtg gtc aag gct tat gcc ttg tcc agc gga gct tcc				1584
Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser				
	515	520	525	
atc att gaa ggt cca ggc ttc acc ggt ggc aac ttg ctc ttc ctt aag				1632
Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys				
	530	535	540	
gag tcc agc aac tcc atc gcc aag ttc aaa gtg aca ctt aac tca gca				1680
Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala				
	545	550	555	560
gcc ttg ctc caa cgt tac agg gtt cgt atc aga tac gca agc act acc				1728
Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr				
	565	570	575	
aat ctt cgc ctc ttt gtc cag aac agc aac aat gat ttc ctt gtc atc				1776
Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile				
	580	585	590	
tac atc aac aag act atg aac aaa gac gat gac ctc acc tac aac aca				1824
Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Asn Thr				
	595	600	605	
ttc gat ctt gcc act acc aat agt aac atg gga ttc tct ggt gac aag				1872
Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys				
	610	615	620	
aac gag ctg atc ata ggt gct gag agc ttt gtc tct aat gag aag att				1920
Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile				
	625	630	635	640
tac ata gac aag atc gag ttc att cca gtt caa ctc taatag				1962
Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu				
	645	650		

<210> 6

<211> 652

<212> PRT

<213> Artificial Sequence

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		20						25					30		

Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met
	35						40					45			

Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp
	50					55				60					

Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val
 65 70 75 80
 Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu
 85 90 95
 Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala
 100 105 110
 Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser
 115 120 125
 Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
 130 135 140
 Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser
 145 150 155 160
 Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser
 165 170 175
 His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu Val
 180 185 190
 Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu
 195 200 205
 Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser Glu
 210 215 220
 Asp Val Ala Glu Phe Tyr His Arg Gln Leu Lys Leu Thr Gln Gln Tyr
 225 230 235 240
 Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu Arg
 245 250 255
 Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu
 260 265 270
 Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr Asp
 275 280 285
 Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp Ile
 290 295 300
 Phe Thr Asp Pro Ile Phe Ser Leu Asn Thr Leu Gln Glu Tyr Gly Pro
 305 310 315 320
 Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe Asp
 325 330 335
 Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr Phe
 340 345 350
 Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr Arg
 355 360 365
 Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly Asp
 370 375 380
 Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys Val
 385 390 395 400

Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly Lys
 405 410 415
 Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp Gln
 420 425 430
 Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly
 435 440 445
 His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr
 450 455 460
 Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu
 465 470 475 480
 Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp
 485 490 495
 Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Asn Thr
 595 600 605
 Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys
 610 615 620
 Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 7

<211> 1989

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: non-naturally
 occurring nucleotide sequence encoding a variant
 Cry3Bb amino acid sequence v11231

<220>

<221> CDS

<222> (3)..(1961)

<223> coding sequence for Cry3Bb variant v11231 amino
 acid sequence

<400> 7

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act cca aac tct gag ttg caa act aat cac aac cag tac cca ttg gct 95
Thr Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala
20 25 30

gac aat cct aac agt act ctt gag gaa ctt aac tac aag gag ttt ctc 143
Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu
35 40 45

cgg atg acc gaa gat agc tcc act gag gtt ctc gat aac tct aca gtg 191
Arg Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val
50 55 60

aag gac gct gtt gga act ggc att agc gtt gtg gga cag att ctt gga 239
Lys Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly
65 70 75

gtg gtt ggt gtt cca ttc gct gga gct ttg acc agc ttc tac cag tcc 287
Val Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser
80 85 90 95

ttt ctc aac acc atc tgg cct tca gat gct gat ccc tgg aag gct ttc 335
Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe
100 105 110

atg gcc caa gtg gaa gtc ttg atc gat aag aag atc gaa gag tat gcc 383
Met Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala
115 120 125

aag tct aaa gcc ttg gct gag ttg caa ggt ttg cag aac aac ttc gag 431
Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu
130 135 140

gat tac gtc aac gca ctc aac agc tgg aag aaa act ccc ttg agt ctc 479
Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu
145 150 155

agg tct aag cgt tcc cag gac cgt att cgt gaa ctt ttc agc caa gcc 527
Arg Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala
160 165 170 175

gaa tcc cac ttc aga aac tcc atg cct agc ttt gcc gtt tct aag ttc 575
Glu Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe
180 185 190

gag gtg ctc ttc ttg cca aca tac gca caa gct gcc aac act cat ctc 623
Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu
195 200 205

ttg ctt ctc aaa gac gct cag gtg ttt ggt gag gaa tgg ggt tac tcc 671
Leu Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser
210 215 220

agt gaa gat gtt gcc gag ttc tac cgt agg cag ctc aag ttg act caa 719
Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln
225 230 235

cag tac aca gac cac tgc gtc aac tgg tac aac gtt ggg ctc aat ggt 767
Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly

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240	245	250	255	
ctt aga gga tct acc tac gac gca tgg gtg aag ttc aac agg ttt cgt Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg	260	265	270	815
aga gag atg acc ttg act gtg ctc gat ctt atc gtt ctc ttt cca ttc Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe	275	280	285	863
tac gac att cgt ctt tac tcc aaa ggc gtt aag aca gag ctg acc aga Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg	290	295	300	911
gac atc ttc acc gat ccc atc ttc cta ctt acg acc ctg cag aaa tac Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr	305	310	315	959
ggt cca act ttt ctc tcc att gag aac agc atc agg aag cct cac ctc Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu	320	325	330	1007
ttc gac tat ctg caa ggc att gag ttt cac acc agg ttg caa cct ggt Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly	340	345	350	1055
tac ttc ggt aag gat tcc ttc aac tac tgg agc gga aac tac gtt gaa Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu	355	360	365	1103
acc aga cca tcc atc gga tct agc aag acc atc act tct cca ttc tac Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr	370	375	380	1151
ggt gac aag agc act gag cca gtg cag aag ttg agc ttc gat ggg cag Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln	385	390	395	1199
aag gtg tat aga acc atc gcc aat acc gat gtt gca gct tgg cct aat Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn	400	405	410	1247
ggc aag gtc tac ctt gga gtt act aaa gtg gac ttc tcc caa tac gac Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp	420	425	430	1295
gat cag aag aac gag aca tct act caa acc tac gat agt aag agg aac Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn	435	440	445	1343
aat ggc cat gtt tcc gca caa gac tcc att gac caa ctt cca cct gaa Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu	450	455	460	1391
acc act gat gaa cca ttg gag aag gct tac agt cac caa ctt aac tac Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr	465	470	475	1439
gcc gaa tgc ttt ctc atg caa gac agg cgt ggc acc att ccg ttc ttt Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe	480	485	490	1487
aca tgg act cac agg tct gtc gac ttc ttt aac act atc gac gct gag Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu				1535

500	505	510	
aag att acc caa ctt ccc gtg gtc aag gct tat gcc ttg tcc agc gga			1583
Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly			
515	520	525	
gct tcc atc att gaa ggt cca ggc ttc acc ggt ggc aac ttg ctc ttc			1631
Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe			
530	535	540	
ctt aag gag tcc agc aac tcc atc gcc aag ttc aaa gtg aca ctt aac			1679
Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn			
545	550	555	
tca gca gcc ttg ctc caa cgt tac agg gtt cgt atc aga tac gca agc			1727
Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser			
560	565	570	575
act acc aat ctt cgc ctc ttt gtc cag aac agc aac aat gat ttc ctt			1775
Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu			
580	585	590	
gtc atc tac atc aac aag act atg aac aaa gac gat gac ctc acc tac			1823
Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr			
595	600	605	
caa aca ttc gat ctt gcc act acc aat agt aac atg gga ttc tct ggt			1871
Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly			
610	615	620	
gac aag aac gag ctg atc ata ggt gct gag agc ttt gtc tct aat gag			1919
Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu			
625	630	635	
aag att tac ata gac aag atc gag ttc att cca gtt caa ctc			1961
Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu			
640	645	650	
taatagatcc cccgggctgc aggaattc			1989

<210> 8

<211> 653

<212> PRT

<213> Artificial Sequence

<400> 8

Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr			
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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp			
20	25	30	
Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg			
35	40	45	
Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys			
50	55	60	
Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val			
65	70	75	80
Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe			
85	90	95	

Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110
 Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125
 Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140
 Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160
 Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
 Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205
 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430

Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445

Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460

Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480

Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495

Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510

Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525

Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540

Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560

Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575

Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590

Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605

Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620

Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640

Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 9

<211> 1984

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: non-naturally
 occurring nucleotide sequence encoding a Cry3Bb
 variant 11231mv1 amino acid sequence

<220>

<221> CDS

<222> (3)..(1961)

<223> coding sequence for a Cry3Bb variant 11231mv1
 amino acid sequence

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 Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val
 1 5 10 15

47

acc ccc aac tcc gag ctc cag acc aac cac aac cag tac ccg ctg gcc	95
Thr Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala	
20 25 30	
gac aac ccc aac tcc acc ctg gaa gag ctg aac tac aag gag ttc ctg	143
Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu	
35 40 45	
cgc atg acc gag gac tcc tcc acg gag gtc ctg gac aac tcc acc gtc	191
Arg Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val	
50 55 60	
aag gac gcc gtc ggg acc ggc atc tcc gtc gtt ggg cag atc ctg ggc	239
Lys Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly	
65 70 75	
gtc gtt ggc gtc ccc ttc gca ggt gct ctc acc tcc ttc tac cag tcc	287
Val Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser	
80 85 90 95	
ttc ctg aac acc atc tgg ccc tcc gac gcc gac ccc tgg aag gcc ttc	335
Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe	
100 105 110	
atg gcc caa gtc gaa gtc ctg atc gac aag aag atc gag gag tac gcc	383
Met Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala	
115 120 125	
aag tcc aag gcc ctg gcc gag ctg caa ggc ctg caa aac aac ttc gag	431
Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu	
130 135 140	
gac tac gtc aac gcg ctg aac tcc tgg aag aag acg cct ctg tcc ctg	479
Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu	
145 150 155	
cgc tcc aag cgc tcc cag ggc cgc atc cgc gag ctg ttc tcc cag gcc	527
Arg Ser Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala	
160 165 170 175	
gag tcc cac ttc cgc aac tcc atg ccg tcc ttc gcc gtc tcc aag ttc	575
Glu Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe	
180 185 190	
gag gtc ctg ttc ctg ccc acc tac gcc cag gct gcc aac acc cac ctc	623
Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu	
195 200 205	
ctg ttg ctg aag gac gcc cag gtc ttc ggc gag gaa tgg ggc tac tcc	671
Leu Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser	
210 215 220	
tcg gag gac gtc gcc gag ttc tac cgt cgc cag ctg aag ctg acc caa	719
Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln	
225 230 235	
cag tac acc gac cac tgc gtc aac tgg tac aac gtc ggc ctg aac ggc	767
Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly	
240 245 250 255	
ctg agg ggc tcc acc tac gac gca tgg gtc aag ttc aac cgc ttc cgc	815
Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg	
260 265 270	

agg gag atg acc ctg acc gtc ctg gac ctg atc gtc ctg ttc ccc ttc	863
Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe	
275 280 285	
tac gac atc cgc ctg tac tcc aag ggc gtc aag acc gag ctg acc cgc	911
Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg	
290 295 300	
gac atc ttc acg gac ccc atc ttc ctg ctc acg acc ctc cag aag tac	959
Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr	
305 310 315	
ggt ccc acc ttc ctg tcc atc gag aac tcc atc cgc aag ccc cac ctg	1007
Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu	
320 325 330 335	
ttc gac tac ctc cag ggc atc gag ttc cac acg cgc ctg agg cca ggc	1055
Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly	
340 345 350	
tac ttc ggc aag gac tcc ttc aac tac tgg tcc ggc aac tac gtc gag	1103
Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu	
355 360 365	
acc agg ccc tcc atc ggc tcc tgc aag acg atc acc tcc cct ttc tac	1151
Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr	
370 375 380	
ggc gac aag tcc acc gag ccc gtc cag aag ctg tcc ttc gac ggc cag	1199
Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln	
385 390 395	
aag gtc tac cgc acc atc gcc aac acc gac gtc gcg gct tgg ccg aac	1247
Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn	
400 405 410 415	
ggc aag gtc tac ctg ggc gtc acg aag gtc gac ttc tcc cag tac gat	1295
Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp	
420 425 430	
gac cag aag aat gaa acc tcc acc cag acc tac gac tcc aag cgc aac	1343
Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn	
435 440 445	
aat ggc cac gtc tcc gcc cag gac tcc atc gac cag ctg ccg cct gag	1391
Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu	
450 455 460	
acc act gac gag ccc ctg gag aag gcc tac tcc cac cag ctg aac tac	1439
Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr	
465 470 475	
gcg gag tgc ttc ctg atg caa gac cgc agg ggc acc atc ccc ttc ttc	1487
Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe	
480 485 490 495	
acc tgg acc cac cgc tcc gtc gac ttc ttc aac acc atc gac gcc gag	1535
Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu	
500 505 510	
aag atc acc cag ctg ccc gtg gtc aag gcc tac gcc ctg tcc tgc ggt	1583
Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly	
515 520 525	

gcc tcc atc att gag ggt cca ggc ttc acc ggt ggc aac ctg ctg ttc 1631
Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe
530 535 540

ctg aag gag tcc tcg aac tcc atc gcc aag ttc aag gtc acc ctg aac 1679
Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn
545 550 555

tcc gct gcc ttg ctg caa cgc tac cgc gtc cgc atc cgc tac gcc tcc 1727
Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser
560 565 570 575

acc acg aac ctg cgc ctg ttc gtc cag aac tcc aac aat gac ttc ctg 1775
Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu
580 585 590

gtc atc tac atc aac aag acc atg aac aag gac gat gac ctg acc tac 1823
Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr
595 600 605

cag acc ttc gac ctc gcc acc acg aac tcc aac atg ggc ttc tcg ggc 1871
Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly
610 615 620

gac aag aat gaa ctg atc att ggt gct gag tcc ttc gtc tcc aat gaa 1919
Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu
625 630 635

aag atc tac atc gac aag atc gag ttc atc ccc gtc cag ctg 1961
Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
640 645 650

tgataggaac tctgattgaa ttc 1984

<210> 10

<211> 653

<212> PRT

<213> Artificial Sequence

<400> 10

Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr
1 5 10 15

Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
20 25 30

Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
35 40 45

Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
50 55 60

Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
65 70 75 80

Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
85 90 95

Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
100 105 110

Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
115 120 125

Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140
 Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160
 Ser Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
 Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205
 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460

Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620
 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640
 Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 11

<211> 1984

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: non-naturally
 occurring nucleotide sequence encoding a Cry3Bb
 variant 11231mv2 amino acid sequence

<220>

<221> CDS

<222> (3)..(1961)

<223> coding sequence for a Cry3Bb variant 11231mv2
 amino acid sequence

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 Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val
 1 5 10 15
 acc ccc aac tcc gag ctg cag acc aac cac aac cag tac ccg ctg gcc 95
 Thr Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala
 20 25 30
 gac aac ccc aac tcc acc ctg gaa gag ctg aac tac aag gag ttc ctg 143
 Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu

35	40	45	
cgc atg acc gag gac tcc tcc acg gag gtc ctg gac aac tcc acc gtc			191
Arg Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val			
50	55	60	
aag gac gcc gtc ggg acc ggc atc tcc gtc gtt ggg cag atc ctg ggc			239
Lys Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly			
65	70	75	
gtc gtt ggc gtc ccc ttc gca ggt gct ctc acc tcc ttc tac cag tcc			287
Val Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser			
80	85	90	95
ttc ctg aac acc atc tgg ccc tcc gac gcc gac ccc tgg aag gcc ttc			335
Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe			
100	105	110	
atg gcc caa gtc gaa gtc ctg atc gac aag aag atc gag gag tac gcc			383
Met Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala			
115	120	125	
aag tcc aag gcc ctg gcc gag ctg caa ggc ctg caa aac aac ttc gag			431
Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu			
130	135	140	
gac tac gtc aac gcg ctg aac tcc tgg aag aag acg cct ctg tcc ctg			479
Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu			
145	150	155	
cgc tcc aag cgc tcc cag gac cgc atc cgc gag ctg ttc tcc cag gcc			527
Arg Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala			
160	165	170	175
gag tcc cac ttc cgc aac tcc atg ccg tcc ttc gcc gtc tcc aag ttc			575
Glu Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe			
180	185	190	
gag gtc ctg ttc ctg ccc acc tac gcc cag gct gcc aac acc cac ctc			623
Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu			
195	200	205	
ctg ttg ctg aag gac gcc cag gtc ttc ggc gag gaa tgg ggc tac tcc			671
Leu Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser			
210	215	220	
tcg gag gac gtc gcc gag ttc tac cgt cgc cag ctg aag ctg acc caa			719
Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln			
225	230	235	
cag tac acc gac cac tgc gtc aac tgg tac aac gtc ggc ctg aac ggc			767
Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly			
240	245	250	255
ctg agg ggc tcc acc tac gac gca tgg gtc aag ttc aac cgc ttc cgc			815
Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg			
260	265	270	
agg gag atg acc ctg acc gtc ctg gac ctg atc gtc ctg ttc ccc ttc			863
Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe			
275	280	285	
tac gac atc cgc ctg tac tcc aag ggc gtc aag acc gag ctg acc cgc			911
Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg			

290	295	300	
gac atc ttc acg gac ccc atc ttc ctg ctc acg acc ctc cag aag tac Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr 305 310 315			959
ggt ccc acc ttc ctg tcc atc gag aac tcc atc cgc aag ccc cac ctg Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu 320 325 330 335			1007
ttc gac tac ctc cag ggc atc gag ttc cac acg cgc ctg agg cca ggc Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly 340 345 350			1055
tac ttc ggc aag gac tcc ttc aac tac tgg tcc ggc aac tac gtc gag Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu 355 360 365			1103
acc agg ccc tcc atc ggc tcc tcg aag acg atc acc tcc cct ttc tac Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr 370 375 380			1151
ggc gac aag tcc acc gag ccc gtc cag aag ctg tcc ttc gac ggc cag Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln 385 390 395			1199
aag gtc tac cgc acc atc gcc aac acc gac gtc gcg gct tgg ccg aac Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn 400 405 410 415			1247
ggc aag gtc tac ctg ggc gtc acg aag gtc gac ttc tcc cag tac gat Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp 420 425 430			1295
gac cag aag aat gaa acc tcc acc cag acc tac gac tcc aag cgc aac Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn 435 440 445			1343
aat ggc cac gtc tcc gcc cag gac tcc atc gac cag ctg ccg cct gag Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu 450 455 460			1391
acc act gac gag ccc ctg gag aag gcc tac tcc cac cag ctg aac tac Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr 465 470 475			1439
gcg gag tgc ttc ctg atg caa gac cgc agg ggc acc atc ccc ttc ttc Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe 480 485 490 495			1487
acc tgg acc cac cgc tcc gtc gac ttc ttc aac acc atc gac gcc gag Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu 500 505 510			1535
aag atc acc cag ctg ccc gtg gtc aag gcc tac gcc ctg tcc tcg ggt Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly 515 520 525			1583
gcc tcc atc att gag ggt cca ggc ttc acc ggt ggc aac ctg ctg ttc Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe 530 535 540			1631
ctg aag gag tcc tcg aac tcc atc gcc aag ttc aag gtc acc ctg aac Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn			1679

545	550	555	
tcc gct gcc ttg ctg caa cgc tac cgc gtc cgc atc cgc tac gcc tcc			1727
Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser			
560	565	570	575
acc acg aac ctg cgc ctg ttc gtc cag aac tcc aac aat gac ttc ctg			1775
Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu			
	580	585	590
gtc atc tac atc aac aag acc atg aac aag gac gat gac ctg acc tac			1823
Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr			
	595	600	605
cag acc ttc gac ctc gcc acc acg aac tcc aac atg ggc ttc tcg ggc			1871
Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly			
	610	615	620
gac aag aat gaa ctg atc att ggt gct gag tcc ttc gtc tcc aat gaa			1919
Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu			
	625	630	635
aag atc tac atc gac aag atc gag ttc atc ccc gtc cag ctg			1961
Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu			
	640	645	650
tgataggaac tctgattgaa ttc			1984
<210> 12			
<211> 653			
<212> PRT			
<213> Artificial Sequence			
<400> 12			
Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr			
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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp			
	20	25	30
Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg			
	35	40	45
Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys			
	50	55	60
Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val			
	65	70	75
Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe			
	85	90	95
Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met			
	100	105	110
Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys			
	115	120	125
Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp			
	130	135	140
Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg			
	145	150	155
			160

Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
 Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205
 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495

Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620
 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640
 Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 13

<211> 4149

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: expression cassette

<220>

<221> promoter

<222> (25)..(640)

<223> P-CaMV.35S

<220>

<221> intron

<222> (669)..(1472)

<223> I-Zm.Hsp70

<220>

<221> transit_peptide

<222> (1489)..(1635)

<223> amino terminal TS-Zm.rbcS

<220>

<221> intron

<222> (1636)..(1798)

<223> I-Zm.rbcS

<220>

<221> transit_peptide

<222> (1799)..(1885)

<223> carboxy terminus TS-Zm.rbcS

<220>
 <221> CDS
 <222> (1885)..(3843)
 <223> Cry3Bb1 variant v11231

<220>
 <221> terminator
 <222> (3871)..(4127)
 <223> T-AGRTu.nos 3' transcription termination and
 polyadenylation sequence

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 ccggaaacct cctcggatcc cattgcccag ctatctgtca ctttattgtg aagatagtgg 120
 aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc atcgttgaag 180
 atgcctctgc cgacagtggc cccaaagatg gacccccacc cacgaggagc atcgtggaaa 240
 aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatggc ccgatgtgag 300
 acttttcaac aaagggtaat atccggaaac ctctcggat tccattgccc agctatctgt 360
 cactttattg tgaagatagt gaaaaggaag gtggctccta caaatgccat cattgcgata 420
 aaggaaaggc catcgttgaa gatgcctctg ccgacagtgg tcccaaagat ggacccccac 480
 ccacgaggag catcgtggaa aaagaagacg tccaaccac gtcttcaaag caagtggatt 540
 gatgtgatat ctccactgac gtaagggatg acgcacaatc ccactatcct tcgcaagacc 600
 ctctctctat ataaggaagt tcatttcatt tggagaggac acgctgacaa gctgactcta 660
 gcagatctac cgtcttcggc acgcgctcac tccgccctct gcctttgtta ctgccacgtt 720
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 actctgaaat cgtgttctgc ctgtgctgat tacttgccgt cctttgtagc agcaaaatat 840
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 ttggtgctta gcggtattta tttaagcaca tgttgggtgt atagggcact tggattcaga 960
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 tattataggc gatactataa taatttgctc gtctgcagag cttattattt gccaaaatta 1080
 gatattccta ttctgttttt gtttgtgtgc tgttaaattg ttaacgcctg aaggaataaa 1140
 tataaatgac gaaattttga tgtttatctc tgctccttta ttgtgaccat aagtcaagat 1200
 cagatgcact tgtttttaa atgtgtgtct gaagaaataa gtactgacag tattttgatg 1260
 cttgatctgc ttgtttgttg taacaaaatt taaaaataaa gagtttcctt tttgttgctc 1320
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 tacgtatctt gctcgatgcc ttctccctag tgttgaccag tgttactcac atagtctttg 1440
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 tgatgatggc ctgctcggcc accgccgtcg ctccgttcct ggggctcaag tccaccgcca 1560

gcctccccgt cgcccgcgcg tcctccagaa gcctcggcaa cgtcagcaac ggcggaagga 1620

tccggtgcat gcaggttaaca aatgcatcct agctagtagt tctttgcatt gcagcagctg 1680

cagctagcga gttagtaata ggaagggaac tgatgatcca tgcattggact gatgtgtgtt 1740

gcccattccca tcccattccca tttcccaaac gaaccgaaaa caccgtacta cgtgcaggtg 1800

tggccctacg gcaacaagaa gttcgagacg ctgtcgtacc tgccgccgct gtcgaccggc 1860

ggcgcatcc gctgcatgca ggcc atg gca aac cct aac aat cgt tcc gaa 1911
Met Ala Asn Pro Asn Asn Arg Ser Glu
1 5

cac gac acc atc aag gtt act cca aac tct gag ttg caa act aat cac 1959
His Asp Thr Ile Lys Val Thr Pro Asn Ser Glu Leu Gln Thr Asn His
10 15 20 25

aac cag tac cca ttg gct gac aat cct aac agt act ctt gag gaa ctt 2007
Asn Gln Tyr Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu
30 35 40

aac tac aag gag ttt ctc cgg atg acc gaa gat agc tcc act gag gtt 2055
Asn Tyr Lys Glu Phe Leu Arg Met Thr Glu Asp Ser Ser Thr Glu Val
45 50 55

ctc gat aac tct aca gtg aag gac gct gtt gga act ggc att agc gtt 2103
Leu Asp Asn Ser Thr Val Lys Asp Ala Val Gly Thr Gly Ile Ser Val
60 65 70

gtg gga cag att ctt gga gtg gtt ggt gtt cca ttc gct gga gct ttg 2151
Val Gly Gln Ile Leu Gly Val Val Gly Val Pro Phe Ala Gly Ala Leu
75 80 85

acc agc ttc tac cag tcc ttt ctc aac acc atc tgg cct tca gat gct 2199
Thr Ser Phe Tyr Gln Ser Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala
90 95 100 105

gat ccc tgg aag gct ttc atg gcc caa gtg gaa gtc ttg atc gat aag 2247
Asp Pro Trp Lys Ala Phe Met Ala Gln Val Glu Val Leu Ile Asp Lys
110 115 120

aag atc gaa gag tat gcc aag tct aaa gcc ttg gct gag ttg caa ggt 2295
Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly
125 130 135

ttg cag aac aac ttc gag gat tac gtc aac gca ctc aac agc tgg aag 2343
Leu Gln Asn Asn Phe Glu Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys
140 145 150

aaa act ccc ttg agt ctc agg tct aag cgt tcc cag gac cgt att cgt 2391
Lys Thr Pro Leu Ser Leu Arg Ser Lys Arg Ser Gln Asp Arg Ile Arg
155 160 165

gaa ctt ttc agc caa gcc gaa tcc cac ttc aga aac tcc atg cct agc 2439
Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser Met Pro Ser
170 175 180 185

ttt gcc gtt tct aag ttc gag gtg ctc ttc ttg cca aca tac gca caa 2487
Phe Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln
190 195 200

gct gcc aac act cat ctc ttg ctt ctc aaa gac gct cag gtg ttt ggt 2535

Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	
			205					210					215			
gag	gaa	tgg	ggt	tac	tcc	agt	gaa	gat	ggt	gcc	gag	ttc	tac	cgt	agg	2583
Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp	Val	Ala	Glu	Phe	Tyr	Arg	Arg	
		220					225					230				
cag	ctc	aag	ttg	act	caa	cag	tac	aca	gac	cac	tgc	gtc	aac	tgg	tac	2631
Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	Thr	Asp	His	Cys	Val	Asn	Trp	Tyr	
		235				240					245					
aac	gtt	ggg	ctc	aat	ggt	ctt	aga	gga	tct	acc	tac	gac	gca	tgg	gtg	2679
Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	Gly	Ser	Thr	Tyr	Asp	Ala	Trp	Val	
250					255					260					265	
aag	ttc	aac	agg	ttt	cgt	aga	gag	atg	acc	ttg	act	gtg	ctc	gat	ctt	2727
Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	
			270					275						280		
atc	gtt	ctc	ttt	cca	ttc	tac	gac	att	cgt	ctt	tac	tcc	aaa	ggc	gtt	2775
Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	Ile	Arg	Leu	Tyr	Ser	Lys	Gly	Val	
			285					290					295			
aag	aca	gag	ctg	acc	aga	gac	atc	ttc	acc	gat	ccc	atc	ttc	cta	ctt	2823
Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	Phe	Thr	Asp	Pro	Ile	Phe	Leu	Leu	
		300					305					310				
acg	acc	ctg	cag	aaa	tac	ggt	cca	act	ttt	ctc	tcc	att	gag	aac	agc	2871
Thr	Thr	Leu	Gln	Lys	Tyr	Gly	Pro	Thr	Phe	Leu	Ser	Ile	Glu	Asn	Ser	
		315				320					325					
atc	agg	aag	cct	cac	ctc	ttc	gac	tat	ctg	caa	ggc	att	gag	ttt	cac	2919
Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	Leu	Gln	Gly	Ile	Glu	Phe	His	
330					335					340					345	
acc	agg	ttg	caa	cct	ggt	tac	ttc	ggt	aag	gat	tcc	ttc	aac	tac	tgg	2967
Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	Gly	Lys	Asp	Ser	Phe	Asn	Tyr	Trp	
			350					355						360		
agc	gga	aac	tac	gtt	gaa	acc	aga	cca	tcc	atc	gga	tct	agc	aag	acc	3015
Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	Pro	Ser	Ile	Gly	Ser	Ser	Lys	Thr	
		365						370					375			
atc	act	tct	cca	ttc	tac	ggt	gac	aag	agc	act	gag	cca	gtg	cag	aag	3063
Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	Lys	Ser	Thr	Glu	Pro	Val	Gln	Lys	
		380				385						390				
ttg	agc	ttc	gat	ggg	cag	aag	gtg	tat	aga	acc	atc	gcc	aat	acc	gat	3111
Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	Tyr	Arg	Thr	Ile	Ala	Asn	Thr	Asp	
		395				400					405					
gtt	gca	gct	tgg	cct	aat	ggc	aag	gtc	tac	ctt	gga	gtt	act	aaa	gtg	3159
Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	
410					415					420					425	
gac	ttc	tcc	caa	tac	gac	gat	cag	aag	aac	gag	aca	tct	act	caa	acc	3207
Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	
			430					435						440		
tac	gat	agt	aag	agg	aac	aat	ggc	cat	gtt	tcc	gca	caa	gac	tcc	att	3255
Tyr	Asp	Ser	Lys	Arg	Asn	Asn	Gly	His	Val	Ser	Ala	Gln	Asp	Ser	Ile	
			445				450						455			
gac	caa	ctt	cca	cct	gaa	acc	act	gat	gaa	cca	ttg	gag	aag	gct	tac	3303

Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr		
		460						465					470				
agt	cac	caa	ctt	aac	tac	gcc	gaa	tgc	ttt	ctc	atg	caa	gac	agg	cgt	3351	
Ser	His	Gln	Leu	Asn	Tyr	Ala	Glu	Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg		
		475						480					485				
ggc	acc	att	ccg	ttc	ttt	aca	tgg	act	cac	agg	tct	gtc	gac	ttc	ttt	3399	
Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	Thr	His	Arg	Ser	Val	Asp	Phe	Phe		
		490						495				500			505		
aac	act	atc	gac	gct	gag	aag	att	acc	caa	ctt	ccc	gtg	gtc	aag	gct	3447	
Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	Thr	Gln	Leu	Pro	Val	Val	Lys	Ala		
				510					515					520			
tat	gcc	ttg	tcc	agc	gga	gct	tcc	atc	att	gaa	ggg	cca	ggc	ttc	acc	3495	
Tyr	Ala	Leu	Ser	Ser	Gly	Ala	Ser	Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr		
			525					530					535				
ggg	ggc	aac	ttg	ctc	ttc	ctt	aag	gag	tcc	agc	aac	tcc	atc	gcc	aag	3543	
Gly	Gly	Asn	Leu	Leu	Phe	Leu	Lys	Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys		
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ttc	aaa	gtg	aca	ctt	aac	tca	gca	gcc	ttg	ctc	caa	cgt	tac	agg	gtt	3591	
Phe	Lys	Val	Thr	Leu	Asn	Ser	Ala	Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val		
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cgt	atc	aga	tac	gca	agc	act	acc	aat	ctt	cgc	ctc	ttt	gtc	cag	aac	3639	
Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn		
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agc	aac	aat	gat	ttc	ctt	gtc	atc	tac	atc	aac	aag	act	atg	aac	aaa	3687	
Ser	Asn	Asn	Asp	Phe	Leu	Val	Ile	Tyr	Ile	Asn	Lys	Thr	Met	Asn	Lys		
				590					595					600			
gac	gat	gac	ctc	acc	tac	caa	aca	ttc	gat	ctt	gcc	act	acc	aat	agt	3735	
Asp	Asp	Asp	Leu	Thr	Tyr	Gln	Thr	Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser		
			605					610					615				
aac	atg	gga	ttc	tct	ggg	gac	aag	aac	gag	ctg	atc	ata	ggg	gct	gag	3783	
Asn	Met	Gly	Phe	Ser	Gly	Asp	Lys	Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu		
		620						625				630					
agc	ttt	gtc	tct	aat	gag	aag	att	tac	ata	gac	aag	atc	gag	ttc	att	3831	
Ser	Phe	Val	Ser	Asn	Glu	Lys	Ile	Tyr	Ile	Asp	Lys	Ile	Glu	Phe	Ile		
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cca	gtt	caa	ctc	taatagatcc	cccggggtgc	aggaattccc	gatcgttcaa									3883	
Pro	Val	Gln	Leu														
				650													
acatttggca	ataaagtttc	ttaagattga	atcctgttgc	cggtcttgcg	atgattatca											3943	
tataatttct	gttgaattac	gttaagcatg	taataattaa	catgtaatgc	atgacgttat											4003	
ttatgagatg	ggtttttatg	attagagtcc	cgcaattata	catttaatac	gcgatagaaa											4063	
acaaaatata	gcgcgcaaac	taggataaat	tatcgcgcg	ggtgtcatct	atgttactag											4123	
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<211> 653

<212> PRT

<213> Artificial Sequence

<400> 14

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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
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Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
 35 40 45

Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
 50 55 60

Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
 65 70 75 80

Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85 90 95

Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110

Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125

Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140

Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160

Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175

Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190

Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205

Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220

Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240

Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255

Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270

Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285

Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300

Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320

Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620
 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640
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 <211> 3754
 <212> DNA
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<220>
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 <222> (25) .. (640)
 <223> P-CaMV.35S

<220>
 <221> intron
 <222> (669) .. (1472)
 <223> I-Zm.Hsp70

<220>
 <221> CDS
 <222> (1490) .. (3448)
 <223> Cry3Bb1 variant v11231

<220>
 <221> terminator
 <222> (3475) .. (3730)
 <223> Agrobacterium tumefaciens nos 3' transcription termination and polyadenylation sequence

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 aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc atcgttgaag 180
 atgcctctgc cgacagtggc cccaaagatg gacccccacc cacgaggagc atcgtggaaa 240
 aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatggc ccatgtgtgag 300
 acttttcaac aaagggtaat atccggaaac ctctcggat tccattgccc agctatctgt 360
 cactttattg tgaagatagt ggaaaaggaa ggtggctcct acaaatgcc tcatgtcgat 420
 aaaggaaagg ccatcgttga agatgcctct gccgacagt gtcccaaaga tggaccccca 480
 cccacgagga gcatcgtgga aaaagaagac gttccaacca cgtcttcaa gcaagtggat 540
 tgatgtgata tctccactga cgtaagggat gacgcacaat cccactatcc ttcgcaagac 600
 ccttcctcta tataagggaag ttcatttcat ttggagagga cacgctgaca agctgactct 660
 agcagatcta ccgtcttcgg tacgcgctca ctccgccctc tgcctttgtt actgccacgt 720
 ttctctgaat gctctcttgt gtggtgattg ctgagagtgg ttagctgga tctagaatta 780
 cactctgaaa tcgtgttctg cctgtgctga ttacttgccg tcctttgtag cagcaaaata 840
 tagggacatg gtagtacgaa acgaagatag aacctacaca gcaatacgag aaatgtgtaa 900
 tttggtgctt agcggtatct atttaagcac atgttggtgt tatagggcac ttggattcag 960
 aagtttgcgt ttaatttagg cacaggcttc atactacatg ggtcaatagt atagggattc 1020

atattatagg cgatactata ataatttgtt cgtctgcaga gcttattatt tgccaaaatt 1080
 agatattcct attctgtttt tgtttgtgtg ctgttaaatt gttaacgcct gaaggaataa 1140
 atataaatga cgaaattttg atgtttatct ctgctccttt attgtgacca taagtcaaga 1200
 tcagatgcac ttgttttaaa tattgttgtc tgaagaaata agtactgaca gtattttgat 1260
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 tgctcatttc attgtaatgc agataccaag cggcctctag aggatctcc atg gca aac 1498
 Met Ala Asn
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 cct aac aat cgt tcc gaa cac gac acc atc aag gtt act cca aac tct 1546
 Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr Pro Asn Ser
 5 10 15
 gag ttg caa act aat cac aac cag tac cca ttg gct gac aat cct aac 1594
 Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn Pro Asn
 20 25 30 35
 agt act ctt gag gaa ctt aac tac aag gag ttt ctc cgg atg acc gaa 1642
 Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met Thr Glu
 40 45 50
 gat agc tcc act gag gtt ctc gat aac tct aca gtg aag gac gct gtt 1690
 Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys Asp Ala Val
 55 60 65
 gga act ggc att agc gtt gtg gga cag att ctt gga gtg gtt ggt gtt 1738
 Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val Gly Val
 70 75 80
 cca ttc gct gga gct ttg acc agc ttc tac cag tcc ttt ctc aac acc 1786
 Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu Asn Thr
 85 90 95
 atc tgg cct tca gat gct gat ccc tgg aag gct ttc atg gcc caa gtg 1834
 Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala Gln Val
 100 105 110 115
 gaa gtc ttg atc gat aag aag atc gaa gag tat gcc aag tct aaa gcc 1882
 Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala
 120 125 130
 ttg gct gag ttg caa ggt ttg cag aac aac ttc gag gat tac gtc aac 1930
 Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val Asn
 135 140 145
 gca ctc aac agc tgg aag aaa act ccc ttg agt ctc agg tct aag cgt 1978
 Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg Ser Lys Arg
 150 155 160
 tcc cag gac cgt att cgt gaa ctt ttc agc caa gcc gaa tcc cac ttc 2026
 Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu Ser His Phe
 165 170 175
 aga aac tcc atg cct agc ttt gcc gtt tct aag ttc gag gtg ctc ttc 2074

Arg 180	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	Leu	Phe	
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ttg	cca	aca	tac	gca	caa	gct	gcc	aac	act	cat	ctc	ttg	ctt	ctc	aaa	2122
Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Leu	Lys	
				200					205					210		
gac	gct	cag	gtg	ttt	ggg	gag	gaa	tgg	ggg	tac	tcc	agt	gaa	gat	gtt	2170
Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp	Val	
			215					220					225			
gcc	gag	ttc	tac	cgt	agg	cag	ctc	aag	ttg	act	caa	cag	tac	aca	gac	2218
Ala	Glu	Phe	Tyr	Arg	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	Thr	Asp	
		230					235					240				
cac	tgc	gtc	aac	tgg	tac	aac	gtt	ggg	ctc	aat	ggg	ctt	aga	gga	tct	2266
His	Cys	Val	Asn	Trp	Tyr	Asn	Val	Gly	Leu	Asn	Gly	Leu	Arg	Gly	Ser	
	245					250					255					
acc	tac	gac	gca	tgg	gtg	aag	ttc	aac	agg	ttt	cgt	aga	gag	atg	acc	2314
Thr	Tyr	Asp	Ala	Trp	Val	Lys	Phe	Asn	Arg	Phe	Arg	Arg	Glu	Met	Thr	
260					265					270					275	
ttg	act	gtg	ctc	gat	ctt	atc	gtt	ctc	ttt	cca	ttc	tac	gac	att	cgt	2362
Leu	Thr	Val	Leu	Asp	Leu	Ile	Val	Leu	Phe	Pro	Phe	Tyr	Asp	Ile	Arg	
				280					285					290		
ctt	tac	tcc	aaa	ggc	gtt	aag	aca	gag	ctg	acc	aga	gac	atc	ttc	acc	2410
Leu	Tyr	Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	Phe	Thr	
			295					300					305			
gat	ccc	atc	ttc	cta	ctt	acg	acc	ctg	cag	aaa	tac	ggg	cca	act	ttt	2458
Asp	Pro	Ile	Phe	Leu	Leu	Thr	Thr	Leu	Gln	Lys	Tyr	Gly	Pro	Thr	Phe	
		310					315					320				
ctc	tcc	att	gag	aac	agc	atc	agg	aag	cct	cac	ctc	ttc	gac	tat	ctg	2506
Leu	Ser	Ile	Glu	Asn	Ser	Ile	Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	Leu	
	325					330					335					
caa	ggc	att	gag	ttt	cac	acc	agg	ttg	caa	cct	ggg	tac	ttc	ggg	aag	2554
Gln	Gly	Ile	Glu	Phe	His	Thr	Arg	Leu	Gln	Pro	Gly	Tyr	Phe	Gly	Lys	
340					345				350					355		
gat	tcc	ttc	aac	tac	tgg	agc	gga	aac	tac	gtt	gaa	acc	aga	cca	tcc	2602
Asp	Ser	Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	Pro	Ser	
			360					365					370			
atc	gga	tct	agc	aag	acc	atc	act	tct	cca	ttc	tac	ggg	gac	aag	agc	2650
Ile	Gly	Ser	Ser	Lys	Thr	Ile	Thr	Ser	Pro	Phe	Tyr	Gly	Asp	Lys	Ser	
		375						380					385			
act	gag	cca	gtg	cag	aag	ttg	agc	ttc	gat	ggg	cag	aag	gtg	tat	aga	2698
Thr	Glu	Pro	Val	Gln	Lys	Leu	Ser	Phe	Asp	Gly	Gln	Lys	Val	Tyr	Arg	
		390				395					400					
acc	atc	gcc	aat	acc	gat	gtt	gca	gct	tgg	cct	aat	ggc	aag	gtc	tac	2746
Thr	Ile	Ala	Asn	Thr	Asp	Val	Ala	Ala	Trp	Pro	Asn	Gly	Lys	Val	Tyr	
	405					410					415					
ctt	gga	gtt	act	aaa	gtg	gac	ttc	tcc	caa	tac	gac	gat	cag	aag	aac	2794
Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp	Gln	Lys	Asn	
420					425					430				435		
gag	aca	tct	act	caa	acc	tac	gat	agt	aag	agg	aac	aat	ggc	cat	gtt	2842

Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn Gly His Val	
440 445 450	
tcc gca caa gac tcc att gac caa ctt cca cct gaa acc act gat gaa	2890
Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu	
455 460 465	
cca ttg gag aag gct tac agt cac caa ctt aac tac gcc gaa tgc ttt	2938
Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys Phe	
470 475 480	
ctc atg caa gac agg cgt ggc acc att ccg ttc ttt aca tgg act cac	2986
Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr His	
485 490 495	
agg tct gtc gac ttc ttt aac act atc gac gct gag aag att acc caa	3034
Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr Gln	
500 505 510 515	
ctt ccc gtg gtc aag gct tat gcc ttg tcc agc gga gct tcc atc att	3082
Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile Ile	
520 525 530	
gaa ggt cca ggc ttc acc ggt ggc aac ttg ctc ttc ctt aag gag tcc	3130
Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser	
535 540 545	
agc aac tcc atc gcc aag ttc aaa gtg aca ctt aac tca gca gcc ttg	3178
Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala Ala Leu	
550 555 560	
ctc caa cgt tac agg gtt cgt atc aga tac gca agc act acc aat ctt	3226
Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu	
565 570 575	
cgc ctc ttt gtc cag aac agc aac aat gat ttc ctt gtc atc tac atc	3274
Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr Ile	
580 585 590 595	
aac aag act atg aac aaa gac gat gac ctc acc tac caa aca ttc gat	3322
Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe Asp	
600 605 610	
ctt gcc act acc aat agt aac atg gga ttc tct ggt gac aag aac gag	3370
Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys Asn Glu	
615 620 625	
ctg atc ata ggt gct gag agc ttt gtc tct aat gag aag att tac ata	3418
Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr Ile	
630 635 640	
gac aag atc gag ttc att cca gtt caa ctc taatagatcc cccgggctgc	3468
Asp Lys Ile Glu Phe Ile Pro Val Gln Leu	
645 650	
aggaattccc gatcgttcaa acatttggca ataaagtttc ttaagattga atcctgttgc	3528
cggctcttgcg atgattatca tataattttct gttgaattac gttaagcatg taataattaa	3588
catgtaatgc atgacgttat ttatgagatg gggtttttatg attagagtcc cgcaattata	3648
catttaatac gcgatagaaa acaaaatata gcgcgcaaac taggataaat tatcgcgcg	3708
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<210> 16
 <211> 653
 <212> PRT
 <213> Artificial Sequence

<400> 16
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 Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
 20 25 30
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 35 40 45
 Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
 50 55 60
 Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
 65 70 75 80
 Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85 90 95
 Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110
 Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125
 Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140
 Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160
 Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
 Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205
 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300

Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
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 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
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 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640

Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
645 650

<210> 17
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<212> DNA
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<220>
<223> Description of Artificial Sequence: expression cassette

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<223> P-CaMV.AS4

<220>
<221> 5'UTR
<222> (240) .. (304)
<223> L-Ta.hcb1

<220>
<221> intron
<222> (318) .. (805)
<223> I-Os.Act1

<220>
<221> transit_peptide
<222> (825) .. (971)
<223> amino terminal TS-Zm.rbcS

<220>
<221> intron
<222> (972) .. (1134)
<223> I-Zm.rbcS

<220>
<221> transit_peptide
<222> (1135) .. (1221)
<223> carboxy terminus TS-Zm.rbcS

<220>
<221> CDS
<222> (1222) .. (3180)
<223> Cry3Bb1 variant 11231mv1

<220>
<221> terminator
<222> (3198) .. (3431)
<223> T-Ta.hsp17

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cactgacgta agggatgacg cacaatccca ctatccttcg caagaccctt cctctatata 180
aggaagttca tttcatttgg agaggacacg ctgacaagct agcttggtg caggtagatc 240
ctagaacat cttccacaca ctcaagccac actattggag aacacacagg gacaacacac 300

cataagatcc aagggaggcc tccgccgccg ccggtaacca ccccgcccct ctctcttttc 360
tttctccggt tttttttccg tctcgggtctc gatctttggc cttggtagtt tgggtgggcg 420
agaggcggtt tctgtgcgcgc ccagatcggg gcgcgggagg ggcgggatct cgcggctggg 480
gctctcgccg gcgtggatcc ggcccggatc tcgcggggaa tggggctctc ggatgtagat 540
ctgcgatccg ccgttggttg gggagatgat ggggggttta aaatttcgc cgtgctaaac 600
aagatcagga agaggggaaa agggcactat gggttatatt tttatatatt tctgtgctt 660
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tccccgtcgc ccgccgctcc tocagaagcc tcggcaacgt cagcaacggc ggaaggatcc 960
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catcccatcc catcccat tccaaacgaa ccgaaaacac cgtactacgt gcaggtgtgg 1140
ccctacggca acaagaagtt cgagacgctg tcgtacctgc cgccgctgct gaccggcggg 1200
cgcatccgct gcatgcaggc c atg gcc aac ccc aac aat cgc tcc gag cac 1251
Met Ala Asn Pro Asn Asn Arg Ser Glu His
1 5 10
gac acg atc aag gtc acc ccc aac tcc gag ctg cag acc aac cac aac 1299
Asp Thr Ile Lys Val Thr Pro Asn Ser Glu Leu Gln Thr Asn His Asn
15 20 25
cag tac ccg ctg gcc gac aac ccc aac tcc acc ctg gaa gag ctg aac 1347
Gln Tyr Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn
30 35 40
tac aag gag ttc ctg cgc atg acc gag gac tcc tcc acg gag gtc ctg 1395
Tyr Lys Glu Phe Leu Arg Met Thr Glu Asp Ser Ser Thr Glu Val Leu
45 50 55
gac aac tcc acc gtc aag gac gcc gtc ggg acc ggc atc tcc gtc gtt 1443
Asp Asn Ser Thr Val Lys Asp Ala Val Gly Thr Gly Ile Ser Val Val
60 65 70
ggg cag atc ctg ggc gtc gtt ggc gtc ccc ttc gca ggt gct ctg acc 1491
Gly Gln Ile Leu Gly Val Val Gly Val Pro Phe Ala Gly Ala Leu Thr
75 80 85 90
tcc ttc tac cag tcc ttc ctg aac acc atc tgg ccc tcc gac gcc gac 1539
Ser Phe Tyr Gln Ser Phe Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp
95 100 105
ccc tgg aag gcc ttc atg gcc caa gtc gaa gtc ctg atc gac aag aag 1587
Pro Trp Lys Ala Phe Met Ala Gln Val Glu Val Leu Ile Asp Lys Lys
110 115 120
atc gag gag tac gcc aag tcc aag gcc ctg gcc gag ctg caa ggc ctg 1635
Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu

125	130	135	
caa aac aac ttc gag gac tac gtc aac gcg ctg aac tcc tgg aag aag Gln Asn Asn Phe Glu Asp Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys 140 145 150			1683
acg cct ctg tcc ctg cgc tcc aag cgc tcc cag ggc cgc atc cgc gag Thr Pro Leu Ser Leu Arg Ser Lys Arg Ser Gln Gly Arg Ile Arg Glu 155 160 165 170			1731
ctg ttc tcc cag gcc gag tcc cac ttc cgc aac tcc atg ccg tcc ttc Leu Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser Met Pro Ser Phe 175 180 185			1779
gcc gtc tcc aag ttc gag gtc ctg ttc ctg ccc acc tac gcc cag gct Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala 190 195 200			1827
gcc aac acc cac ctc ctg ttg ctg aag gac gcc cag gtc ttc ggc gag Ala Asn Thr His Leu Leu Leu Leu Lys Asp Ala Gln Val Phe Gly Glu 205 210 215			1875
gaa tgg ggc tac tcc tcg gag gac gtc gcc gag ttc tac cgt cgc cag Glu Trp Gly Tyr Ser Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln 220 225 230			1923
ctg aag ctg acc caa cag tac acc gac cac tgc gtc aac tgg tac aac Leu Lys Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn 235 240 245 250			1971
gtc ggc ctg aac ggc ctg agg ggc tcc acc tac gac gca tgg gtc aag Val Gly Leu Asn Gly Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys 255 260 265			2019
ttc aac cgc ttc cgc agg gag atg acc ctg acc gtc ctg gac ctg atc Phe Asn Arg Phe Arg Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile 270 275 280			2067
gtc ctg ttc ccc ttc tac gac atc cgc ctg tac tcc aag ggc gtc aag Val Leu Phe Pro Phe Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys 285 290 295			2115
acc gag ctg acc cgc gac atc ttc acg gac ccc atc ttc ctg ctc acg Thr Glu Leu Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr 300 305 310			2163
acc ctc cag aag tac ggt ccc acc ttc ctg tcc atc gag aac tcc atc Thr Leu Gln Lys Tyr Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile 315 320 325 330			2211
cgc aag ccc cac ctg ttc gac tac ctc cag ggc atc gag ttc cac acg Arg Lys Pro His Leu Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr 335 340 345			2259
cgc ctg agg cca ggc tac ttc ggc aag gac tcc ttc aac tac tgg tcc Arg Leu Arg Pro Gly Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser 350 355 360			2307
ggc aac tac gtc gag acc agg ccc tcc atc ggc tcc tcg aag acg atc Gly Asn Tyr Val Glu Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile 365 370 375			2355
acc tcc cct ttc tac ggc gac aag tcc acc gag ccc gtc cag aag ctg Thr Ser Pro Phe Tyr Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu			2403

380	385	390	
tcc ttc gac ggc cag aag gtc tac cgc acc atc gcc aac acc gac gtc Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val 395 400 405 410	2451		
gcg gct tgg ccg aac ggc aag gtc tac ctg ggc gtc acg aag gtc gac Ala Ala Trp Pro Asn Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp 415 420 425	2499		
ttc tcc cag tac gat gac cag aag aat gaa acc tcc acc cag acc tac Phe Ser Gln Tyr Asp Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr 430 435 440	2547		
gac tcc aag cgc aac aat ggc cac gtc tcc gcc cag gac tcc atc gac Asp Ser Lys Arg Asn Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp 445 450 455	2595		
cag ctg ccg cct gag acc act gac gag ccc ctg gag aag gcc tac tcc Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser 460 465 470	2643		
cac cag ctg aac tac gcg gag tgc ttc ctg atg caa gac cgc agg ggc His Gln Leu Asn Tyr Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly 475 480 485 490	2691		
acc atc ccc ttc ttc acc tgg acc cac cgc tcc gtc gac ttc ttc aac Thr Ile Pro Phe Phe Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn 495 500 505	2739		
acc atc gac gcc gag aag atc acc cag ctg ccc gtg gtc aag gcc tac Thr Ile Asp Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr 510 515 520	2787		
gcc ctg tcc tcg ggt gcc tcc atc att gag ggt cca ggc ttc acc ggt Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly 525 530 535	2835		
ggc aac ctg ctg ttc ctg aag gag tcc tcg aac tcc atc gcc aag ttc Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe 540 545 550	2883		
aag gtc acc ctg aac tcc gct gcc ttg ctg caa cgc tac cgc gtc cgc Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg 555 560 565 570	2931		
atc cgc tac gcc tcc acc acg aac ctg cgc ctg ttc gtc cag aac tcc Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser 575 580 585	2979		
aac aat gac ttc ctg gtc atc tac atc aac aag acc atg aac aag gac Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp 590 595 600	3027		
gat gac ctg acc tac cag acc ttc gac ctc gcc acc acg aac tcc aac Asp Asp Leu Thr Tyr Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn 605 610 615	3075		
atg ggc ttc tcg ggc gac aag aat gaa ctg atc att ggt gct gag tcc Met Gly Phe Ser Gly Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser 620 625 630	3123		
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			20					25					30			
Asn	Pro	Asn	Ser	Thr	Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	
		35				40						45				
Met	Thr	Glu	Asp	Ser	Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	
	50					55					60					
Asp	Ala	Val	Gly	Thr	Gly	Ile	Ser	Val	Val	Gly	Gln	Ile	Leu	Gly	Val	
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Val	Gly	Val	Pro	Phe	Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	
				85					90					95		
Leu	Asn	Thr	Ile	Trp	Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	
			100					105					110			
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		115					120					125				
Ser	Lys	Ala	Leu	Ala	Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	
	130					135					140					
Tyr	Val	Asn	Ala	Leu	Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	
145					150					155					160	
Ser	Lys	Arg	Ser	Gln	Gly	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	
				165					170					175		
Ser	His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	
			180					185					190			
Val	Leu	Phe	Leu	Pro	Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	
		195					200					205				
Leu	Leu	Lys	Asp	Ala	Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	
	210					215					220					
Glu	Asp	Val	Ala	Glu	Phe	Tyr	Arg	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	
225					230					235					240	

Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
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 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
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 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
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 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
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 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
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 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575

Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590

Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605

Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620

Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
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Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
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<210> 19

<211> 3039

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: expression cassette

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<222> (14) .. (235)

<223> P-CaMV.AS4

<220>

<221> 5'UTR

<222> (240) .. (304)

<223> L-Ta.hcb1

<220>

<221> intron

<222> (318) .. (805)

<223> I-Os.Act1

<220>

<221> CDS

<222> (811) .. (2769)

<223> Cry3Bb1 variant 11231mv1

<220>

<221> terminator

<222> (2787) .. (3020)

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cactgacgta agggatgacg cacaatccca ctatccttcg caagaccctt cctctatata 180

aggaagttca tttcatttgg agaggacacg ctgacaagct agcttggtg caggtagatc 240

ctagaaccat cttccacaca ctcaagccac actattggag aacacacagg gacaacacac 300

cataagatcc aagggaggcc tccgcccgcg ccggtaacca ccccgcccct ctctcttttc 360

tttctccggt tttttttccg tctcggtctc gatctttggc cttggtagtt tgggtgggcg 420

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ctgcgatccg ccgttggttg gggagatgat ggggggttta aaatttcgc cgtgctaaac 600
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gagctttttt gtaggtagaa gtgatcaacc atg gcc aac ccc aac aat cgc tcc 834
                               Met Ala Asn Pro Asn Asn Arg Ser
                               1               5

gag cac gac acg atc aag gtc acc ccc aac tcc gag ctg cag acc aac 882
Glu His Asp Thr Ile Lys Val Thr Pro Asn Ser Glu Leu Gln Thr Asn
    10               15               20

cac aac cag tac ccg ctg gcc gac aac ccc aac tcc acc ctg gaa gag 930
His Asn Gln Tyr Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu
    25               30               35               40

ctg aac tac aag gag ttc ctg cgc atg acc gag gac tcc tcc acg gag 978
Leu Asn Tyr Lys Glu Phe Leu Arg Met Thr Glu Asp Ser Ser Thr Glu
                45               50               55

gtc ctg gac aac tcc acc gtc aag gac gcc gtc ggg acc ggc atc tcc 1026
Val Leu Asp Asn Ser Thr Val Lys Asp Ala Val Gly Thr Gly Ile Ser
                60               65               70

gtc gtt ggg cag atc ctg ggc gtc gtt ggc gtc ccc ttc gca ggt gct 1074
Val Val Gly Gln Ile Leu Gly Val Val Gly Val Pro Phe Ala Gly Ala
                75               80               85

ctc acc tcc ttc tac cag tcc ttc ctg aac acc atc tgg ccc tcc gac 1122
Leu Thr Ser Phe Tyr Gln Ser Phe Leu Asn Thr Ile Trp Pro Ser Asp
    90               95               100

gcc gac ccc tgg aag gcc ttc atg gcc caa gtc gaa gtc ctg atc gac 1170
Ala Asp Pro Trp Lys Ala Phe Met Ala Gln Val Glu Val Leu Ile Asp
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Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu Ala Glu Leu Gln
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Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val Asn Ala Leu Asn Ser Trp
                140               145               150

aag aag acg cct ctg tcc ctg cgc tcc aag cgc tcc cag ggc cgc atc 1314
Lys Lys Thr Pro Leu Ser Leu Arg Ser Lys Arg Ser Gln Gly Arg Ile
                155               160               165

cgc gag ctg ttc tcc cag gcc gag tcc cac ttc cgc aac tcc atg ccg 1362
Arg Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser Met Pro
    170               175               180

tcc ttc gcc gtc tcc aag ttc gag gtc ctg ttc ctg ccc acc tac gcc 1410
Ser Phe Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro Thr Tyr Ala
   185               190               195               200

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cag gct gcc aac acc cac ctc ctg ttg ctg aag gac gcc cag gtc ttc Gln Ala Ala Asn Thr His Leu Leu Leu Leu Lys Asp Ala Gln Val Phe 205 210 215	1458
ggc gag gaa tgg ggc tac tcc tcg gag gac gtc gcc gag ttc tac cgt Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp Val Ala Glu Phe Tyr Arg 220 225 230	1506
cgc cag ctg aag ctg acc caa cag tac acc gac cac tgc gtc aac tgg Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp 235 240 245	1554
tac aac gtc ggc ctg aac ggc ctg agg ggc tcc acc tac gac gca tgg Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly Ser Thr Tyr Asp Ala Trp 250 255 260	1602
gtc aag ttc aac cgc ttc cgc agg gag atg acc ctg acc gtc ctg gac Val Lys Phe Asn Arg Phe Arg Arg Glu Met Thr Leu Thr Val Leu Asp 265 270 275 280	1650
ctg atc gtc ctg ttc ccc ttc tac gac atc cgc ctg tac tcc aag ggc Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile Arg Leu Tyr Ser Lys Gly 285 290 295	1698
gtc aag acc gag ctg acc cgc gac atc ttc acg gac ccc atc ttc ctg Val Lys Thr Glu Leu Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Leu 300 305 310	1746
ctc acg acc ctc cag aag tac ggt ccc acc ttc ctg tcc atc gag aac Leu Thr Thr Leu Gln Lys Tyr Gly Pro Thr Phe Leu Ser Ile Glu Asn 315 320 325	1794
tcc atc cgc aag ccc cac ctg ttc gac tac ctc cag ggc atc gag ttc Ser Ile Arg Lys Pro His Leu Phe Asp Tyr Leu Gln Gly Ile Glu Phe 330 335 340	1842
cac acg cgc ctg agg cca ggc tac ttc ggc aag gac tcc ttc aac tac His Thr Arg Leu Arg Pro Gly Tyr Phe Gly Lys Asp Ser Phe Asn Tyr 345 350 355 360	1890
tgg tcc ggc aac tac gtc gag acc agg ccc tcc atc ggc tcc tcg aag Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro Ser Ile Gly Ser Ser Lys 365 370 375	1938
acg atc acc tcc cct ttc tac ggc gac aag tcc acc gag ccc gtc cag Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys Ser Thr Glu Pro Val Gln 380 385 390	1986
aag ctg tcc ttc gac ggc cag aag gtc tac cgc acc atc gcc aac acc Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile Ala Asn Thr 395 400 405	2034
gac gtc gcg gct tgg ccg aac ggc aag gtc tac ctg ggc gtc acg aag Asp Val Ala Ala Trp Pro Asn Gly Lys Val Tyr Leu Gly Val Thr Lys 410 415 420	2082
gtc gac ttc tcc cag tac gat gac cag aag aat gaa acc tcc acc cag Val Asp Phe Ser Gln Tyr Asp Asp Gln Lys Asn Glu Thr Ser Thr Gln 425 430 435 440	2130
acc tac gac tcc aag cgc aac aat ggc cac gtc tcc gcc cag gac tcc Thr Tyr Asp Ser Lys Arg Asn Asn Gly His Val Ser Ala Gln Asp Ser 445 450 455	2178

atc gac cag ctg ccg cct gag acc act gac gag ccc ctg gag aag gcc 2226
 Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu Lys Ala
 460 465 470

tac tcc cac cag ctg aac tac gcg gag tgc ttc ctg atg caa gac cgc 2274
 Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys Phe Leu Met Gln Asp Arg
 475 480 485

agg ggc acc atc ccc ttc ttc acc tgg acc cac cgc tcc gtc gac ttc 2322
 Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr His Arg Ser Val Asp Phe
 490 495 500

ttc aac acc atc gac gcc gag aag atc acc cag ctg ccc gtg gtc aag 2370
 Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys
 505 510 515 520

gcc tac gcc ctg tcc tcg ggt gcc tcc atc att gag ggt cca ggc ttc 2418
 Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly Phe
 525 530 535

acc ggt ggc aac ctg ctg ttc ctg aag gag tcc tcg aac tcc atc gcc 2466
 Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn Ser Ile Ala
 540 545 550

aag ttc aag gtc acc ctg aac tcc gct gcc ttg ctg caa cgc tac cgc 2514
 Lys Phe Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln Arg Tyr Arg
 555 560 565

gtc cgc atc cgc tac gcc tcc acc acg aac ctg cgc ctg ttc gtc cag 2562
 Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu Phe Val Gln
 570 575 580

aac tcc aac aat gac ttc ctg gtc atc tac atc aac aag acc atg aac 2610
 Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr Met Asn
 585 590 595 600

aag gac gat gac ctg acc tac cag acc ttc gac ctc gcc acc acg aac 2658
 Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe Asp Leu Ala Thr Thr Asn
 605 610 615

tcc aac atg ggc ttc tcg ggc gac aag aat gaa ctg atc att ggt gct 2706
 Ser Asn Met Gly Phe Ser Gly Asp Lys Asn Glu Leu Ile Ile Gly Ala
 620 625 630

gag tcc ttc gtc tcc aat gaa aag atc tac atc gac aag atc gag ttc 2754
 Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr Ile Asp Lys Ile Glu Phe
 635 640 645

atc ccc gtc cag ctg tgataggaac tctgattgaa ttctgcatgc gtttggacgt 2809
 Ile Pro Val Gln Leu
 650

atgctcattc aggttggagc caatttggtt gatgtgtgtg cgagtctctg cgagtctgat 2869

gagacatctc tgtatttgtt ttctttcccc agtggtttct gtacttgtgt aatcggttaa 2929

tcgccaacag attcggcgat gaataaatga gaaataaatt gttctgattt tgagtgcaaa 2989

aaaaaaggaa ttagatctgt gtgtgttttt tggatccccg gggcggccgc 3039

<210> 20

<211> 653

<212> PRT

<213> Artificial Sequence

<400> 20

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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
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Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
 35 40 45

Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
 50 55 60

Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
 65 70 75 80

Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85 90 95

Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110

Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125

Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140

Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160

Ser Lys Arg Ser Gln Gly Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175

Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190

Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205

Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220

Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240

Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255

Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270

Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285

Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300

Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320

Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620
 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640
 Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 21
 <211> 3039
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: expression cassette

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<221> promoter
 <222> (14)..(235)
 <223> P-CaMV.AS4

<220>

<221> 5'UTR
 <222> (240)..(304)
 <223> L-Ta.hcb1

<220>

<221> intron
 <222> (318)..(805)
 <223> I-Os.Act1

<220>

<221> CDS
 <222> (811)..(2769)
 <223> Cry3Bb1 variant 11231mv2

<220>

<221> terminator
 <222> (2787)..(3020)
 <223> T-Ta.hsp17

<400> 21

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acctgacgta agggatgacg cacctgacgt aagggatgac gcactcgaga tccccatctc 120
cactgacgta agggatgacg cacaatccca ctatccttcg caagaccctt cctctatata 180
aggaagtcca tttcatttgg agaggacacg ctgacaagct agcttggttg caggtagatc 240
ctagaacccat cttccacaca ctcaagccac actattggag aacacacagg gacaacacac 300
cataagatcc aaggaggagg tccgcgcgcg ccggtaacca ccccgccctt ctctcttttc 360
tttctcgcgt ttttttttcg tctcggtctc gatctttggc cttggtagtt tgggtgggcg 420
agaggcggct tcgtgcgcgc ccagatcggg gcgcgggagg ggcgggatct cgcggctggg 480
gctctcgcgc gcgtggatcc ggcccgatc tcgcggggaa tggggctctc ggatgtagat 540
ctgcgatccg ccgttggttg gggagatgat ggggggttta aaatttcgc cgtgctaaac 600
aagatcagga agaggggaaa agggcactat ggtttatatt tttatatatt tctgctgctt 660
cgtcaggctt agatgtgcta gatctttctt tcttcttttt gtgggtagaa tttgaatccc 720
tcagcattgt tcatcggtag tttttctttt catgatttgt gacaaatgca gcctcgtgcg 780
gagctttttt gtaggtagaa gtgatcaacc atg gcc aac ccc aac aat cgc tcc 834
Met Ala Asn Pro Asn Asn Arg Ser
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gag cac gac acg atc aag gtc acc ccc aac tcc gag ctc cag acc aac	882
Glu His Asp Thr Ile Lys Val Thr Pro Asn Ser Glu Leu Gln Thr Asn	
10 15 20	
cac aac cag tac ccg ctg gcc gac aac ccc aac tcc acc ctg gaa gag	930
His Asn Gln Tyr Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu	
25 30 35 40	
ctg aac tac aag gag ttc ctg cgc atg acc gag gac tcc tcc acg gag	978
Leu Asn Tyr Lys Glu Phe Leu Arg Met Thr Glu Asp Ser Ser Thr Glu	
45 50 55	
gtc ctg gac aac tcc acc gtc aag gac gcc gtc ggg acc ggc atc tcc	1026
Val Leu Asp Asn Ser Thr Val Lys Asp Ala Val Gly Thr Gly Ile Ser	
60 65 70	
gtc gtt ggg cag atc ctg ggc gtc gtt ggc gtc ccc ttc gca ggt gct	1074
Val Val Gly Gln Ile Leu Gly Val Val Gly Val Pro Phe Ala Gly Ala	
75 80 85	
ctc acc tcc ttc tac cag tcc ttc ctg aac acc atc tgg ccc tcc gac	1122
Leu Thr Ser Phe Tyr Gln Ser Phe Leu Asn Thr Ile Trp Pro Ser Asp	
90 95 100	
gcc gac ccc tgg aag gcc ttc atg gcc caa gtc gaa gtc ctg atc gac	1170
Ala Asp Pro Trp Lys Ala Phe Met Ala Gln Val Glu Val Leu Ile Asp	
105 110 115 120	
aag aag atc gag gag tac gcc aag tcc aag gcc ctg gcc gag ctg caa	1218
Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu Ala Glu Leu Gln	
125 130 135	
ggc ctg caa aac aac ttc gag gac tac gtc aac gcg ctg aac tcc tgg	1266
Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val Asn Ala Leu Asn Ser Trp	
140 145 150	
aag aag acg cct ctg tcc ctg cgc tcc aag cgc tcc cag gac cgc atc	1314
Lys Lys Thr Pro Leu Ser Leu Arg Ser Lys Arg Ser Gln Asp Arg Ile	
155 160 165	
cgc gag ctg ttc tcc cag gcc gag tcc cac ttc cgc aac tcc atg ccg	1362
Arg Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser Met Pro	
170 175 180	
tcc ttc gcc gtc tcc aag ttc gag gtc ctg ttc ctg ccc acc tac gcc	1410
Ser Phe Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro Thr Tyr Ala	
185 190 195 200	
cag gct gcc aac acc cac ctc ctg ttg ctg aag gac gcc cag gtc ttc	1458
Gln Ala Ala Asn Thr His Leu Leu Leu Lys Asp Ala Gln Val Phe	
205 210 215	
ggc gag gaa tgg ggc tac tcc tcg gag gac gtc gcc gag ttc tac cgt	1506
Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp Val Ala Glu Phe Tyr Arg	
220 225 230	
cgc cag ctg aag ctg acc caa cag tac acc gac cac tgc gtc aac tgg	1554
Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp	
235 240 245	
tac aac gtc ggc ctg aac ggc ctg agg ggc tcc acc tac gac gca tgg	1602
Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly Ser Thr Tyr Asp Ala Trp	
250 255 260	

gtc aag ttc aac cgc ttc cgc agg gag atg acc ctg acc gtc ctg gac	1650
Val Lys Phe Asn Arg Phe Arg Arg Glu Met Thr Leu Thr Val Leu Asp	
265 270 275 280	
ctg atc gtc ctg ttc ccc ttc tac gac atc cgc ctg tac tcc aag ggc	1698
Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile Arg Leu Tyr Ser Lys Gly	
285 290 295	
gtc aag acc gag ctg acc cgc gac atc ttc acg gac ccc atc ttc ctg	1746
Val Lys Thr Glu Leu Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Leu	
300 305 310	
ctc acg acc ctc cag aag tac ggt ccc acc ttc ctg tcc atc gag aac	1794
Leu Thr Thr Leu Gln Lys Tyr Gly Pro Thr Phe Leu Ser Ile Glu Asn	
315 320 325	
tcc atc cgc aag ccc cac ctg ttc gac tac ctc cag ggc atc gag ttc	1842
Ser Ile Arg Lys Pro His Leu Phe Asp Tyr Leu Gln Gly Ile Glu Phe	
330 335 340	
cac acg cgc ctg agg cca ggc tac ttc ggc aag gac tcc ttc aac tac	1890
His Thr Arg Leu Arg Pro Gly Tyr Phe Gly Lys Asp Ser Phe Asn Tyr	
345 350 355 360	
tgg tcc ggc aac tac gtc gag acc agg ccc tcc atc ggc tcc tcg aag	1938
Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro Ser Ile Gly Ser Ser Lys	
365 370 375	
acg atc acc tcc cct ttc tac ggc gac aag tcc acc gag ccc gtc cag	1986
Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys Ser Thr Glu Pro Val Gln	
380 385 390	
aag ctg tcc ttc gac ggc cag aag gtc tac cgc acc atc gcc aac acc	2034
Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile Ala Asn Thr	
395 400 405	
gac gtc gcg gct tgg ccg aac ggc aag gtc tac ctg ggc gtc acg aag	2082
Asp Val Ala Ala Trp Pro Asn Gly Lys Val Tyr Leu Gly Val Thr Lys	
410 415 420	
gtc gac ttc tcc cag tac gat gac cag aag aat gaa acc tcc acc cag	2130
Val Asp Phe Ser Gln Tyr Asp Asp Gln Lys Asn Glu Thr Ser Thr Gln	
425 430 435 440	
acc tac gac tcc aag cgc aac aat ggc cac gtc tcc gcc cag gac tcc	2178
Thr Tyr Asp Ser Lys Arg Asn Asn Gly His Val Ser Ala Gln Asp Ser	
445 450 455	
atc gac cag ctg ccg cct gag acc act gac gag ccc ctg gag aag gcc	2226
Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu Lys Ala	
460 465 470	
tac tcc cac cag ctg aac tac gcg gag tgc ttc ctg atg caa gac cgc	2274
Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys Phe Leu Met Gln Asp Arg	
475 480 485	
agg ggc acc atc ccc ttc ttc acc tgg acc cac cgc tcc gtc gac ttc	2322
Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr His Arg Ser Val Asp Phe	
490 495 500	
ttc aac acc atc gac gcc gag aag atc acc cag ctg ccc gtg gtc aag	2370
Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys	
505 510 515 520	

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gcc tac gcc ctg tcc tcg ggt gcc tcc atc att gag ggt cca ggc ttc 2418
Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly Phe
      525                      530                      535

acc ggt ggc aac ctg ctg ttc ctg aag gag tcc tcg aac tcc atc gcc 2466
Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn Ser Ile Ala
      540                      545                      550

aag ttc aag gtc acc ctg aac tcc gct gcc ttg ctg caa cgc tac cgc 2514
Lys Phe Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln Arg Tyr Arg
      555                      560                      565

gtc cgc atc cgc tac gcc tcc acc acg aac ctg cgc ctg ttc gtc cag 2562
Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu Phe Val Gln
      570                      575                      580

aac tcc aac aat gac ttc ctg gtc atc tac atc aac aag acc atg aac 2610
Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr Met Asn
      585                      590                      595                      600

aag gac gat gac ctg acc tac cag acc ttc gac ctc gcc acc acg aac 2658
Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe Asp Leu Ala Thr Thr Asn
      605                      610                      615

tcc aac atg ggc ttc tcg ggc gac aag aat gaa ctg atc att ggt gct 2706
Ser Asn Met Gly Phe Ser Gly Asp Lys Asn Glu Leu Ile Ile Gly Ala
      620                      625                      630

gag tcc ttc gtc tcc aat gaa aag atc tac atc gac aag atc gag ttc 2754
Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr Ile Asp Lys Ile Glu Phe
      635                      640                      645

atc ccc gtc cag ctg tgataggaac tctgattgaa ttctgcatgc gtttggacgt 2809
Ile Pro Val Gln Leu
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atgctcattc aggttggagc caatttggtt gatgtgtgtg cgagttcttg cgagtctgat 2869

gagacatctc tgtatttgtt ttctttcccc agtgttttct gtacttgtgt aatcggctaa 2929

tcgccaacag attcggcgat gaataaatga gaaataaatt gttctgattt tgagtgcaaa 2989

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<210> 22

<211> 653

<212> PRT

<213> Artificial Sequence

<400> 22

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Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr
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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
      20              25              30

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Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
      35              40              45

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Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
      50              55              60

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Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
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 Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85 90 95
 Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110
 Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125
 Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140
 Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160
 Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
 Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205
 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly

405																410				415			
Lys	Val	Tyr	Leu	Gly	Val	Thr	Lys	Val	Asp	Phe	Ser	Gln	Tyr	Asp	Asp								
420								425				430											
Gln	Lys	Asn	Glu	Thr	Ser	Thr	Gln	Thr	Tyr	Asp	Ser	Lys	Arg	Asn	Asn								
435								440				445											
Gly	His	Val	Ser	Ala	Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr								
450								455				460											
Thr	Asp	Glu	Pro	Leu	Glu	Lys	Ala	Tyr	Ser	His	Gln	Leu	Asn	Tyr	Ala								
465				470								475				480							
Glu	Cys	Phe	Leu	Met	Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr								
				485				490								495							
Trp	Thr	His	Arg	Ser	Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys								
500								505				510											
Ile	Thr	Gln	Leu	Pro	Val	Val	Lys	Ala	Tyr	Ala	Leu	Ser	Ser	Gly	Ala								
515								520				525											
Ser	Ile	Ile	Glu	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asn	Leu	Leu	Phe	Leu								
530				535								540											
Lys	Glu	Ser	Ser	Asn	Ser	Ile	Ala	Lys	Phe	Lys	Val	Thr	Leu	Asn	Ser								
545				550				555				560											
Ala	Ala	Leu	Leu	Gln	Arg	Tyr	Arg	Val	Arg	Ile	Arg	Tyr	Ala	Ser	Thr								
				565				570				575											
Thr	Asn	Leu	Arg	Leu	Phe	Val	Gln	Asn	Ser	Asn	Asn	Asp	Phe	Leu	Val								
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Thr	Phe	Asp	Leu	Ala	Thr	Thr	Asn	Ser	Asn	Met	Gly	Phe	Ser	Gly	Asp								
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Lys	Asn	Glu	Leu	Ile	Ile	Gly	Ala	Glu	Ser	Phe	Val	Ser	Asn	Glu	Lys								
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 $\langle 220 \rangle$

<223> Description of Artificial Sequence: expression cassette

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$\langle 220 \rangle$

<221> 5'UTR

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<222> (748)..(1238)

<223> I-Os.Act1

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<221> CDS

<222> (1241)..(3199)

<223> Cry3Bb1 variant 11231mv2

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<222> (3217)..(3450)

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aat cgc tcc gag cac gac acg atc aag gtc acc ccc aac tcc gag ctc 1303

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Gln	Thr	Asn	His	Asn	Gln	Tyr	Pro	Leu	Ala	Asp	Asn	Pro	Asn	Ser	Thr	
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Leu	Glu	Glu	Leu	Asn	Tyr	Lys	Glu	Phe	Leu	Arg	Met	Thr	Glu	Asp	Ser	
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Ser	Thr	Glu	Val	Leu	Asp	Asn	Ser	Thr	Val	Lys	Asp	Ala	Val	Gly	Thr	
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Gly	Ile	Ser	Val	Val	Gln	Ile	Leu	Gly	Val	Val	Gly	Val	Pro	Phe		
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Ala	Gly	Ala	Leu	Thr	Ser	Phe	Tyr	Gln	Ser	Phe	Leu	Asn	Thr	Ile	Trp	
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Pro	Ser	Asp	Ala	Asp	Pro	Trp	Lys	Ala	Phe	Met	Ala	Gln	Val	Glu	Val	
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ctg	atc	gac	aag	aag	atc	gag	gag	tac	gcc	aag	tcc	aag	gcc	ctg	gcc	1639
Leu	Ile	Asp	Lys	Lys	Ile	Glu	Glu	Tyr	Ala	Lys	Ser	Lys	Ala	Leu	Ala	
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gag	ctg	caa	ggc	ctg	caa	aac	aac	ttc	gag	gac	tac	gtc	aac	gcg	ctg	1687
Glu	Leu	Gln	Gly	Leu	Gln	Asn	Asn	Phe	Glu	Asp	Tyr	Val	Asn	Ala	Leu	
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Asn	Ser	Trp	Lys	Lys	Thr	Pro	Leu	Ser	Leu	Arg	Ser	Lys	Arg	Ser	Gln	
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Asp	Arg	Ile	Arg	Glu	Leu	Phe	Ser	Gln	Ala	Glu	Ser	His	Phe	Arg	Asn	
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Ser	Met	Pro	Ser	Phe	Ala	Val	Ser	Lys	Phe	Glu	Val	Leu	Phe	Leu	Pro	
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Thr	Tyr	Ala	Gln	Ala	Ala	Asn	Thr	His	Leu	Leu	Leu	Lys	Asp	Ala		
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Gln	Val	Phe	Gly	Glu	Glu	Trp	Gly	Tyr	Ser	Ser	Glu	Asp	Val	Ala	Glu	
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Phe	Tyr	Arg	Arg	Gln	Leu	Lys	Leu	Thr	Gln	Gln	Tyr	Thr	Asp	His	Cys	
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Ser	Lys	Gly	Val	Lys	Thr	Glu	Leu	Thr	Arg	Asp	Ile	Phe	Thr	Asp	Pro		
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Ile	Phe	Leu	Leu	Thr	Thr	Leu	Gln	Lys	Tyr	Gly	Pro	Thr	Phe	Leu	Ser		
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Phe	Asn	Tyr	Trp	Ser	Gly	Asn	Tyr	Val	Glu	Thr	Arg	Pro	Ser	Ile	Gly		
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Ala	Asn	Thr	Asp	Val	Ala	Trp	Pro	Asn	Gly	Lys	Val	Tyr	Leu	Gly			
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Gln	Asp	Ser	Ile	Asp	Gln	Leu	Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro	Leu		
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Gln	Asp	Arg	Arg	Gly	Thr	Ile	Pro	Phe	Phe	Thr	Trp	Thr	His	Arg	Ser		
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Val	Asp	Phe	Phe	Asn	Thr	Ile	Asp	Ala	Glu	Lys	Ile	Thr	Gln	Leu	Pro		
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Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly
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cca ggc ttc acc ggt ggc aac ctg ctg ttc ctg aag gag tcc tcg aac 2887
Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn
535 540 545

tcc atc gcc aag ttc aag gtc acc ctg aac tcc gct gcc ttg ctg caa 2935
Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln
550 555 560 565

cgc tac cgc gtc cgc atc cgc tac gcc tcc acc acg aac ctg cgc ctg 2983
Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu
570 575 580

ttc gtc cag aac tcc aac aat gac ttc ctg gtc atc tac atc aac aag 3031
Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys
585 590 595

acc atg aac aag gac gat gac ctg acc tac cag acc ttc gac ctc gcc 3079
Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe Asp Leu Ala
600 605 610

acc acg aac tcc aac atg ggc ttc tcg ggc gac aag aat gaa ctg atc 3127
Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp Lys Asn Glu Leu Ile
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att ggt gct gag tcc ttc gtc tcc aat gaa aag atc tac atc gac aag 3175
Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr Ile Asp Lys
630 635 640 645

atc gag ttc atc ccc gtc cag ctg tgataggaac tctgattgaa ttctgcatgc 3229
Ile Glu Phe Ile Pro Val Gln Leu
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35 40 45
Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
50 55 60
Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
65 70 75 80

Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85 90 95
 Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110
 Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125
 Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
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 Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160
 Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
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 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415

Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
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 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
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 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
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<211> 416

<212> DNA

<213> Artificial Sequence

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 ribulose bis-phosphate carboxylase chloroplast
 targeting peptide

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<222> (16)..(162)

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<223> I-Zm.rbcS

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Ala Val Ala Pro Phe Leu Gly Leu Lys Ser Thr Ala Ser Leu Pro Val

15

20

25

gcc cgc cgc tcc tcc aga agc ctc ggc aac gtc agc aac ggc gga agg 147

Ala Arg Arg Ser Ser Arg Ser Leu Gly Asn Val Ser Asn Gly Gly Arg

30

35

40

atc cgg tgc atg cag gtaacaaatg catcctagct agtagttctt tgcattgcag 202

Ile Arg Cys Met Gln

45

cagctgcagc tagcgagtta gtaataggaa gggaactgat gatccatgca tggactgatg 262

tgtgttgccc atcccattccc atcccatttc ccaaacgaac cgaaaacacc gtactacgtg 322

cag gtg tgg ccc tac ggc aac aag aag ttc gag acg ctg tcg tac ctg 370

Val Trp Pro Tyr Gly Asn Lys Lys Phe Glu Thr Leu Ser Tyr Leu

50

55

60

ccg ccg ctg tcg acc ggc ggg cgc atc cgc tgc atg cag gcc atg g 416

Pro Pro Leu Ser Thr Gly Gly Arg Ile Arg Cys Met Gln Ala Met

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<212> PRT

<213> Artificial Sequence

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Phe Leu Gly Leu Lys Ser Thr Ala Ser Leu Pro Val Ala Arg Arg Ser

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25

30

Ser Arg Ser Leu Gly Asn Val Ser Asn Gly Gly Arg Ile Arg Cys Met

35

40

45

Gln Val Trp Pro Tyr Gly Asn Lys Lys Phe Glu Thr Leu Ser Tyr Leu

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Pro Pro Leu Ser Thr Gly Gly Arg Ile Arg Cys Met Gln Ala Met

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<210> 28
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 <211> 202
 <212> DNA
 <213> Cauliflower mosaic virus

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 <212> DNA
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 <223> Description of Artificial Sequence: modified
 cauliflower mosaic virus promoter AS4

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 ctcggcaacg tcagcaacgg cggaaggatc cgggtgcatgc aggttaacaaa tgcattcctag 180
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 atgatccatg catggactga tgtgtgttgc ccattccatc ccattccatt tcccaaacga 300
 accgaaaaca ccgtactacg tgcaggtgtg gccctacggc aacaagaagt tcgagacgct 360
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 <212> DNA
 <213> Triticum aestivum

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<210> 32
 <211> 804
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 <213> Oryza sp.

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 atcgtgttct gcctgtgctg attacttgcc gtcctttgta gcagcaaaat atagggacat 180
 ggtagtacga aacgaagata gaacctacac agcaatacga gaaatgtgta atttgggtgct 240
 tagcgggtatt tatttaagca catgttggtg ttataggga cttggattca gaagtttgct 300
 gttaatttag gcacaggctt catactacat gggtaaatag tatagggatt catattatag 360
 gcgatactat aataatttgt tcgtctgcag agcttattat ttgccaaaat tagatattcc 420
 tattctgttt ttgtttgtgt gctgttaaat tgttaacgcc tgaaggaata aatataaatg 480
 acgaaatatt gatgtttatc tctgctcctt tattgtgacc ataagtcaag atcagatgca 540
 cttgttttaa atattgttgt ctgaagaaat aagtactgac agtatatttga tgcattgac 600
 tgcttggttg ttgtaacaaa atttaaaaat aaagagtttc ctttttggtg ctctccttac 660
 ctctgatgg tatctagtat ctaccaactg acactatatt gcttctcttt acatacgtat 720
 cttgctcgat gccttctccc tagtggtgac cagtgttact cacatagtct ttgctcattt 780
 cattgtaatg cagataccaa gcgg 804

<210> 33
 <211> 804
 <212> DNA
 <213> Zea mays

<400> 33
 accgtcttcg gtacgcgctc actccgccct ctgcctttgt tactgccacg tttctctgaa 60
 tgctctcttg tgtgggtgatt gctgagagtg gtttagctgg atctagaatt acactctgaa 120
 atcgtgttct gcctgtgctg attacttgcc gtcctttgta gcagcaaaat atagggacat 180
 ggtagtacga aacgaagata gaacctacac agcaatacga gaaatgtgta atttgggtgct 240
 tagcgggtatt tatttaagca catgttggtg ttataggga cttggattca gaagtttgct 300
 gttaatttag gcacaggctt catactacat gggtaaatag tatagggatt catattatag 360

gcgatactat aataatTTgt tCgtctgcag agcttattat ttgccaaaat tagatattcc 420
 tattctgttt ttgtttgtgt gctgttaaT tgttaacgcc tgaaggaata aatataaatg 480
 acgaaatTTt gatgtttatc tctgtctcctt tattgtgacc ataagtcaag atcagatgca 540
 cttgttttaa atattgttgt ctgaagaaT aagtactgac agtattttga tgcattgatc 600
 tgcttgTTtg ttgtaacaaa atttaaaaaT aaagagtTtc ctttttgTtg ctctccttac 660
 ctctgatgg tatctagtat ctaccaactg acactatatt gcttctcttt acatacgtat 720
 cttgtctgat gccttctccc tagtgTtgac cagtgttact cacatagtct ttgtcattt 780
 cattgtaatg cagataccaa gcgg 804

<210> 34
 <211> 257
 <212> DNA
 <213> Agrobacterium tumefaciens

<400> 34
 tcccgatcgt tcaaacattt ggcaataaag tttcttaaga ttgaatcctg ttgccggTct 60
 tgcgatgatt atcatataat ttctgttgaa ttacgttaag catgtaataa ttaacatgta 120
 atgcatgacg ttatttatga gatgggTttt tatgattaga gtcccgcaat tatacattta 180
 atacgcgata gaaaacaaaa tatagcgcgc aaactaggat aaattatcgc gcgcggTgtc 240
 atctatgtta ctagatc 257

<210> 35
 <211> 234
 <212> DNA
 <213> Triticum aestivum

<400> 35
 aattctgcat gcgTttggac gtatgctcat tcaggTtga gccaatTtg ttgatgtgtg 60
 tgcgagTtct tgcgagtctg atgagacatc tctgtattgt gtttctttcc ccagtgtttt 120
 ctgtacttgt gtaatcggt aatcgccaac agattcggcg atgaataaT gagaaataa 180
 ttgttctgat ttgagtgc aaaaaaagg aattagatct gtgtgtgttt ttg 234

<210> 36
 <211> 3455
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: expression
 cassette

<220>
 <221> promoter
 <222> (14) .. (235)
 <223> P.CaMV.AS4

<220>
 <221> 5'UTR
 <222> (240)..(304)
 <223> L-Ta.hcb1

 <220>
 <221> intron
 <222> (318)..(805)
 <223> I-Os.Act1

 <220>
 <221> transit_peptide
 <222> (825)..(971)
 <223> TS-Zm.rbcS amino terminal coding sequence upstream
 of Zea mays rbcS intron

 <220>
 <221> intron
 <222> (972)..(1134)
 <223> I-Zm.rbcS

 <220>
 <221> transit_peptide
 <222> (1135)..(1221)
 <223> TS-Zm.rbcS carboxy terminus coding sequence
 downstream of Zea mays rbcS intron

 <220>
 <221> CDS
 <222> (1222)..(3180)
 <223> variant Cry3BB1 coding sequence encoding v11231

 <220>
 <221> terminator
 <222> (3198)..(3431)
 <223> T-Ta.hsp17

<400> 36
 gcgggccgcgt taacaagctt ctgacgtaag ggatgacgca cctgacgtaa gggatgacgc 60

 acctgacgta agggatgacg cacctgacgt aaggatgac gactcgaga tccccatctc 120

 cactgacgta agggatgacg cacaatccca ctatccttcg caagaccctt cctctatata 180

 aggaagttca tttcatttgg agaggacacg ctgacaagct agcttggtg caggtagatc 240

 ctagaaccat cttccacaca ctcaagccac actattggag aacacacagg gacaacacac 300

 cataagatcc aaggaggagg tccgcccgcg ccggtaacca ccccgcccct ctctctttc 360

 tttctccgtt tttttttccg tctcggtctc gatctttggc cttggtagtt tgggtgggcg 420

 agaggcggct tcgtgcgcgc ccagatcggg gcgcgggagg ggcgggatct cgcggctggg 480

 gctctcgccg gcgtggatcc ggcccggatc tcgcggggaa tggggtctc ggatgtagat 540

 ctgcgatccg ccgttggttg gggagatgat ggggggttta aaatttcgc cgtgctaaac 600

 aagatcagga agaggggaaa agggcactat ggtttatatt tttatatatt tctgctgctt 660

 cgtcaggctt agatgtgcta gatctttctt tcttcttttt gtgggtagaa tttgaatccc 720

 tcagcattgt tcatcggtag tttttctttt catgatttgt gacaaatgca gcctcgtgcg 780

72

190	195	200	
gcc aac act cat ctc ttg ctt ctc aaa gac gct cag gtg ttt ggt gag Ala Asn Thr His Leu Leu Leu Leu Lys Asp Ala Gln Val Phe Gly Glu 205 210 215			1875
gaa tgg ggt tac tcc agt gaa gat gtt gcc gag ttc tac cgt agg cag Glu Trp Gly Tyr Ser Ser Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln 220 225 230			1923
ctc aag ttg act caa cag tac aca gac cac tgc gtc aac tgg tac aac Leu Lys Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp Tyr Asn 235 240 245 250			1971
gtt ggg ctc aat ggt ctt aga gga tct acc tac gac gca tgg gtg aag Val Gly Leu Asn Gly Leu Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys 255 260 265			2019
ttc aac agg ttt cgt aga gag atg acc ttg act gtg ctc gat ctt atc Phe Asn Arg Phe Arg Arg Glu Met Thr Leu Thr Val Leu Asp Leu Ile 270 275 280			2067
gtt ctc ttt cca ttc tac gac att cgt ctt tac tcc aaa ggc gtt aag Val Leu Phe Pro Phe Tyr Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys 285 290 295			2115
aca gag ctg acc aga gac atc ttc acc gat ccc atc ttc cta ctt acg Thr Glu Leu Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr 300 305 310			2163
acc ctg cag aaa tac ggt cca act ttt ctc tcc att gag aac agc atc Thr Leu Gln Lys Tyr Gly Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile 315 320 325 330			2211
agg aag cct cac ctc ttc gac tat ctg caa ggc att gag ttt cac acc Arg Lys Pro His Leu Phe Asp Tyr Leu Gln Gly Ile Glu Phe His Thr 335 340 345			2259
agg ttg caa cct ggt tac ttc ggt aag gat tcc ttc aac tac tgg agc Arg Leu Gln Pro Gly Tyr Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser 350 355 360			2307
gga aac tac gtt gaa acc aga cca tcc atc gga tct agc aag acc atc Gly Asn Tyr Val Glu Thr Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile 365 370 375			2355
act tct cca ttc tac ggt gac aag agc act gag cca gtg cag aag ttg Thr Ser Pro Phe Tyr Gly Asp Lys Ser Thr Glu Pro Val Gln Lys Leu 380 385 390			2403
agc ttc gat ggg cag aag gtg tat aga acc atc gcc aat acc gat gtt Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile Ala Asn Thr Asp Val 395 400 405 410			2451
gca gct tgg cct aat ggc aag gtc tac ctt gga gtt act aaa gtg gac Ala Ala Trp Pro Asn Gly Lys Val Tyr Leu Gly Val Thr Lys Val Asp 415 420 425			2499
ttc tcc caa tac gac gat cag aag aac gag aca tct act caa acc tac Phe Ser Gln Tyr Asp Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr 430 435 440			2547
gat agt aag agg aac aat ggc cat gtt tcc gca caa gac tcc att gac Asp Ser Lys Arg Asn Asn Gly His Val Ser Ala Gln Asp Ser Ile Asp			2595

445	450	455	
caa ctt cca cct gaa acc act gat gaa cca ttg gag aag gct tac agt Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser 460 465 470			2643
cac caa ctt aac tac gcc gaa tgc ttt ctc atg caa gac agg cgt ggc His Gln Leu Asn Tyr Ala Glu Cys Phe Leu Met Gln Asp Arg Arg Gly 475 480 485 490			2691
acc att ccg ttc ttt aca tgg act cac agg tct gtc gac ttc ttt aac Thr Ile Pro Phe Phe Thr Trp Thr His Arg Ser Val Asp Phe Phe Asn 495 500 505			2739
act atc gac gct gag aag att acc caa ctt ccc gtg gtc aag gct tat Thr Ile Asp Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr 510 515 520			2787
gcc ttg tcc agc gga gct tcc atc att gaa ggt cca ggc ttc acc ggt Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly 525 530 535			2835
ggc aac ttg ctc ttc ctt aag gag tcc agc aac tcc atc gcc aag ttc Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe 540 545 550			2883
aaa gtg aca ctt aac tca gca gcc ttg ctc caa cgt tac agg gtt cgt Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg 555 560 565 570			2931
atc aga tac gca agc act acc aat ctt cgc ctc ttt gtc cag aac agc Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser 575 580 585			2979
aac aat gat ttc ctt gtc atc tac atc aac aag act atg aac aaa gac Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp 590 595 600			3027
gat gac ctc acc tac caa aca ttc gat ctt gcc act acc aat agt aac Asp Asp Leu Thr Tyr Gln Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn 605 610 615			3075
atg gga ttc tct ggt gac aag aac gag ctg atc ata ggt gct gag agc Met Gly Phe Ser Gly Asp Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser 620 625 630			3123
ttt gtc tct aat gag aag att tac ata gac aag atc gag ttc att cca Phe Val Ser Asn Glu Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro 635 640 645 650			3171
gtt caa ctc taatagatcc cccgggctgc aggaattctg catgcgtttg Val Gln Leu			3220
gacgtatgct cattcaggtt ggagccaatt tggttgatgt gtgtgagagt tcttgagagt			3280
ctgatgagac atctctgtat tgtgtttctt tccccagtgt tttctgtact tgtgtaatcg			3340
gctaatcgcc aacagattcg gcgatgaata aatgagaaat aaattgttct gatttttgagt			3400
gcaaaaaaaaa aggaattaga tctgtgtgtg ttttttggat ccccggggag gccgc			3455

<210> 37

<211> 653

<212> PRT

<213> Artificial Sequence

<400> 37

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Met Ala Asn Pro Asn Asn Arg Ser Glu His Asp Thr Ile Lys Val Thr
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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
      20             25             30

Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
      35             40             45

Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
      50             55             60

Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
      65             70             75             80

Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
      85             90             95

Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
      100            105            110

Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
      115            120            125

Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
      130            135            140

Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
      145            150            155            160

Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
      165            170            175

Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
      180            185            190

Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
      195            200            205

Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
      210            215            220

Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
      225            230            235            240

Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
      245            250            255

Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
      260            265            270

Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
      275            280            285

Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
      290            295            300

Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
      305            310            315            320

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Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
 Asp Lys Ser Thr Glu Pro Val Gln Lys Leu Ser Phe Asp Gly Gln Lys
 385 390 395 400
 Val Tyr Arg Thr Ile Ala Asn Thr Asp Val Ala Ala Trp Pro Asn Gly
 405 410 415
 Lys Val Tyr Leu Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp Asp
 420 425 430
 Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser Lys Arg Asn Asn
 435 440 445
 Gly His Val Ser Ala Gln Asp Ser Ile Asp Gln Leu Pro Pro Glu Thr
 450 455 460
 Thr Asp Glu Pro Leu Glu Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala
 465 470 475 480
 Glu Cys Phe Leu Met Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr
 485 490 495
 Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys
 500 505 510
 Ile Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala
 515 520 525
 Ser Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu
 530 535 540
 Lys Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser
 545 550 555 560
 Ala Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr
 565 570 575
 Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val
 580 585 590
 Ile Tyr Ile Asn Lys Thr Met Asn Lys Asp Asp Asp Leu Thr Tyr Gln
 595 600 605
 Thr Phe Asp Leu Ala Thr Thr Asn Ser Asn Met Gly Phe Ser Gly Asp
 610 615 620
 Lys Asn Glu Leu Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys
 625 630 635 640
 Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln Leu
 645 650

<210> 38
 <211> 3044
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: expression cassette

<220>

<221> promoter
 <222> (14) .. (235)
 <223> P-CaMV.AS4

<220>

<221> 5'UTR
 <222> (240) .. (304)
 <223> L-Ta.hcb1

<220>

<221> intron
 <222> (318) .. (805)
 <223> I-Os.Act1

<220>

<221> CDS
 <222> (811) .. (2769)
 <223> variant Cry3Bb1 coding sequence encoding v11231

<220>

<221> terminator
 <222> (2792) .. (3025)
 <223> T-Ta.hsp17

<400> 38

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cactgacgta agggatgacg cacaatccca ctatccttcg caagaccctt cctctatata 180
aggaagtcca tttcatttgg agaggacacg ctgacaagct agcttggtcg caggtagatc 240
ctagaaccat cttccacaca ctcaagccac actattggag aacacacagg gacaacacac 300
cataagatcc aaggaggacc tccgccgccg ccggtaacca ccccgccctt ctctcttttc 360
tttctccgtt tttttttccg tctcggtctc gatctttggc cttggtagtt tgggtgggcg 420
agaggcggtt tcgtgcgcgc ccagatcggt gcgcgggagg ggcgggatct cgcggctggg 480
gctctcgccg gcgtggatcc ggcccggatc tcgcggggaa tggggctctc ggatgtagat 540
ctgcgatccg ccgttggttg gggagatgat ggggggttta aaatttccgc cgtgctaaac 600
aagatcagga agaggggaaa agggcactat ggtttatatt tttatatatt tctgctgctt 660
cgtcaggctt agatgtgcta gatctttctt tcttcttttt gtgggtagaa tttgaatccc 720
tcagcattgt tcatcggtag tttttctttt catgatttgt gacaaatgca gcctcgtagc 780
gagctttttt gtaggtagaa gtgatcaacc atg gca aac cct aac aat cgt tcc 834
Met Ala Asn Pro Asn Asn Arg Ser

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gaa cac gac acc atc aag gtt act cca aac tct gag ttg caa act aat	882
Glu His Asp Thr Ile Lys Val Thr Pro Asn Ser Glu Leu Gln Thr Asn	
10 15 20	
cac aac cag tac cca ttg gct gac aat cct aac agt act ctt gag gaa	930
His Asn Gln Tyr Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu	
25 30 35 40	
ctt aac tac aag gag ttt ctc cgg atg acc gaa gat agc tcc act gag	978
Leu Asn Tyr Lys Glu Phe Leu Arg Met Thr Glu Asp Ser Ser Thr Glu	
45 50 55	
gtt ctc gat aac tct aca gtg aag gac gct gtt gga act ggc att agc	1026
Val Leu Asp Asn Ser Thr Val Lys Asp Ala Val Gly Thr Gly Ile Ser	
60 65 70	
gtt gtg gga cag att ctt gga gtg gtt ggt gtt cca ttc gct gga gct	1074
Val Val Gly Gln Ile Leu Gly Val Val Gly Val Pro Phe Ala Gly Ala	
75 80 85	
ttg acc agc ttc tac cag tcc ttt ctc aac acc atc tgg cct tca gat	1122
Leu Thr Ser Phe Tyr Gln Ser Phe Leu Asn Thr Ile Trp Pro Ser Asp	
90 95 100	
gct gat ccc tgg aag gct ttc atg gcc caa gtg gaa gtc ttg atc gat	1170
Ala Asp Pro Trp Lys Ala Phe Met Ala Gln Val Glu Val Leu Ile Asp	
105 110 115 120	
aag aag atc gaa gag tat gcc aag tct aaa gcc ttg gct gag ttg caa	1218
Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu Ala Glu Leu Gln	
125 130 135	
ggt ttg cag aac aac ttc gag gat tac gtc aac gca ctc aac agc tgg	1266
Gly Leu Gln Asn Asn Phe Glu Asp Tyr Val Asn Ala Leu Asn Ser Trp	
140 145 150	
aag aaa act ccc ttg agt ctc agg tct aag cgt tcc cag gac cgt att	1314
Lys Lys Thr Pro Leu Ser Leu Arg Ser Lys Arg Ser Gln Asp Arg Ile	
155 160 165	
cgt gaa ctt ttc agc caa gcc gaa tcc cac ttc aga aac tcc atg cct	1362
Arg Glu Leu Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser Met Pro	
170 175 180	
agc ttt gcc gtt tct aag ttc gag gtg ctc ttc ttg cca aca tac gca	1410
Ser Phe Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro Thr Tyr Ala	
185 190 195 200	
caa gct gcc aac act cat ctc ttg ctt ctc aaa gac gct cag gtg ttt	1458
Gln Ala Ala Asn Thr His Leu Leu Leu Leu Lys Asp Ala Gln Val Phe	
205 210 215	
ggt gag gaa tgg ggt tac tcc agt gaa gat gtt gcc gag ttc tac cgt	1506
Gly Glu Glu Trp Gly Tyr Ser Ser Glu Asp Val Ala Glu Phe Tyr Arg	
220 225 230	
agg cag ctc aag ttg act caa cag tac aca gac cac tgc gtc aac tgg	1554
Arg Gln Leu Lys Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp	
235 240 245	
tac aac gtt ggg ctc aat ggt ctt aga gga tct acc tac gac gca tgg	1602
Tyr Asn Val Gly Leu Asn Gly Leu Arg Gly Ser Thr Tyr Asp Ala Trp	
250 255 260	

gtg aag ttc aac agg ttt cgt aga gag atg acc ttg act gtg ctc gat	1650
Val Lys Phe Asn Arg Phe Arg Arg Glu Met Thr Leu Thr Val Leu Asp	
265 270 275 280	
ctt atc gtt ctc ttt cca ttc tac gac att cgt ctt tac tcc aaa ggc	1698
Leu Ile Val Leu Phe Pro Phe Tyr Asp Ile Arg Leu Tyr Ser Lys Gly	
285 290 295	
gtt aag aca gag ctg acc aga gac atc ttc acc gat ccc atc ttc cta	1746
Val Lys Thr Glu Leu Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Leu	
300 305 310	
ctt acg acc ctg cag aaa tac ggt cca act ttt ctc tcc att gag aac	1794
Leu Thr Thr Leu Gln Lys Tyr Gly Pro Thr Phe Leu Ser Ile Glu Asn	
315 320 325	
agc atc agg aag cct cac ctc ttc gac tat ctg caa ggc att gag ttt	1842
Ser Ile Arg Lys Pro His Leu Phe Asp Tyr Leu Gln Gly Ile Glu Phe	
330 335 340	
cac acc agg ttg caa cct ggt tac ttc ggt aag gat tcc ttc aac tac	1890
His Thr Arg Leu Gln Pro Gly Tyr Phe Gly Lys Asp Ser Phe Asn Tyr	
345 350 355 360	
tgg agc gga aac tac gtt gaa acc aga cca tcc atc gga tct agc aag	1938
Trp Ser Gly Asn Tyr Val Glu Thr Arg Pro Ser Ile Gly Ser Ser Lys	
365 370 375	
acc atc act tct cca ttc tac ggt gac aag agc act gag cca gtg cag	1986
Thr Ile Thr Ser Pro Phe Tyr Gly Asp Lys Ser Thr Glu Pro Val Gln	
380 385 390	
aag ttg agc ttc gat ggg cag aag gtg tat aga acc atc gcc aat acc	2034
Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile Ala Asn Thr	
395 400 405	
gat gtt gca gct tgg cct aat ggc aag gtc tac ctt gga gtt act aaa	2082
Asp Val Ala Ala Trp Pro Asn Gly Lys Val Tyr Leu Gly Val Thr Lys	
410 415 420	
gtg gac ttc tcc caa tac gac gat cag aag aac gag aca tct act caa	2130
Val Asp Phe Ser Gln Tyr Asp Asp Gln Lys Asn Glu Thr Ser Thr Gln	
425 430 435 440	
acc tac gat agt aag agg aac aat ggc cat gtt tcc gca caa gac tcc	2178
Thr Tyr Asp Ser Lys Arg Asn Asn Gly His Val Ser Ala Gln Asp Ser	
445 450 455	
att gac caa ctt cca cct gaa acc act gat gaa cca ttg gag aag gct	2226
Ile Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu Lys Ala	
460 465 470	
tac agt cac caa ctt aac tac gcc gaa tgc ttt ctc atg caa gac agg	2274
Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys Phe Leu Met Gln Asp Arg	
475 480 485	
cgt ggc acc att ccg ttc ttt aca tgg act cac agg tct gtc gac ttc	2322
Arg Gly Thr Ile Pro Phe Phe Thr Trp Thr His Arg Ser Val Asp Phe	
490 495 500	
ttt aac act atc gac gct gag aag att acc caa ctt ccc gtg gtc aag	2370
Phe Asn Thr Ile Asp Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys	
505 510 515 520	

gct tat gcc ttg tcc agc gga gct tcc atc att gaa ggt cca ggc ttc 2418
Ala Tyr Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly Phe
525 530 535

acc ggt ggc aac ttg ctc ttc ctt aag gag tcc agc aac tcc atc gcc 2466
Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser Asn Ser Ile Ala
540 545 550

aag ttc aaa gtg aca ctt aac tca gca gcc ttg ctc caa cgt tac agg 2514
Lys Phe Lys Val Thr Leu Asn Ser Ala Ala Leu Leu Gln Arg Tyr Arg
555 560 565

gtt cgt atc aga tac gca agc act acc aat ctt cgc ctc ttt gtc cag 2562
Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Arg Leu Phe Val Gln
570 575 580

aac agc aac aat gat ttc ctt gtc atc tac atc aac aag act atg aac 2610
Asn Ser Asn Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr Met Asn
585 590 595 600

aaa gac gat gac ctc acc tac caa aca ttc gat ctt gcc act acc aat 2658
Lys Asp Asp Asp Leu Thr Tyr Gln Thr Phe Asp Leu Ala Thr Thr Asn
605 610 615

agt aac atg gga ttc tct ggt gac aag aac gag ctg atc ata ggt gct 2706
Ser Asn Met Gly Phe Ser Gly Asp Lys Asn Glu Leu Ile Ile Gly Ala
620 625 630

gag agc ttt gtc tct aat gag aag att tac ata gac aag atc gag ttc 2754
Glu Ser Phe Val Ser Asn Glu Lys Ile Tyr Ile Asp Lys Ile Glu Phe
635 640 645

att cca gtt caa ctc taatagatcc cccgggctgc aggaattctg catgcgtttg 2809
Ile Pro Val Gln Leu
650

gacgtatgct cattcagggtt ggagccaatt tggttgatgt gtgtgagagt tcttgagagt 2869

ctgatgagac atctctgtat tgtgtttctt tccccagtgt tttctgtact tgtgtaatcg 2929

gctaatacgcc aacagattcg gcgatgaata aatgagaaat aaattgttct gattttgagt 2989

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<211> 653

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Pro Asn Ser Glu Leu Gln Thr Asn His Asn Gln Tyr Pro Leu Ala Asp
20 25 30

Asn Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg
35 40 45

Met Thr Glu Asp Ser Ser Thr Glu Val Leu Asp Asn Ser Thr Val Lys
50 55 60

Asp Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val
 65 70 75 80
 Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe
 85 90 95
 Leu Asn Thr Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met
 100 105 110
 Ala Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys
 115 120 125
 Ser Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp
 130 135 140
 Tyr Val Asn Ala Leu Asn Ser Trp Lys Lys Thr Pro Leu Ser Leu Arg
 145 150 155 160
 Ser Lys Arg Ser Gln Asp Arg Ile Arg Glu Leu Phe Ser Gln Ala Glu
 165 170 175
 Ser His Phe Arg Asn Ser Met Pro Ser Phe Ala Val Ser Lys Phe Glu
 180 185 190
 Val Leu Phe Leu Pro Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu
 195 200 205
 Leu Leu Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser Ser
 210 215 220
 Glu Asp Val Ala Glu Phe Tyr Arg Arg Gln Leu Lys Leu Thr Gln Gln
 225 230 235 240
 Tyr Thr Asp His Cys Val Asn Trp Tyr Asn Val Gly Leu Asn Gly Leu
 245 250 255
 Arg Gly Ser Thr Tyr Asp Ala Trp Val Lys Phe Asn Arg Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr
 275 280 285
 Asp Ile Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu Thr Arg Asp
 290 295 300
 Ile Phe Thr Asp Pro Ile Phe Leu Leu Thr Thr Leu Gln Lys Tyr Gly
 305 310 315 320
 Pro Thr Phe Leu Ser Ile Glu Asn Ser Ile Arg Lys Pro His Leu Phe
 325 330 335
 Asp Tyr Leu Gln Gly Ile Glu Phe His Thr Arg Leu Gln Pro Gly Tyr
 340 345 350
 Phe Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val Glu Thr
 355 360 365
 Arg Pro Ser Ile Gly Ser Ser Lys Thr Ile Thr Ser Pro Phe Tyr Gly
 370 375 380
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oligonucleotide

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<210> 43

<211> 25

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<223> Description of Artificial Sequence: synthetic
oligonucleotide

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cgagttctac cgtaggcagc tcaag

25